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The University of Alberta

Doctoral Thesis

THE ROLE OF CAROTID-SINUS-NERVE AFFERENTS AND SYMPATHETIC EFFERENTS IN THE REGULATION OF BRONCHOMOTOR TONE IN GUINEA PIGS

Submitted by

Sudhir J.A. D'Souza

In partial fulfilment of the Degree of

Doctor of Philosophy

in

Pharmaceutical Sciences (Pharmacology)

Faculty of Pharmacy and Pharmaceutical Sciences

Edmonton, Alberta

Spring 1990

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ISBN 0-315-70147-1

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DEGREE FOR WHICH THESIS WAS PRESENTED **DOCTOR OF PHILOSOPHY**

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **THE ROLE OF CAROTID SINUS NERVE AFFERENTS AND SYMPATHETIC EFFERENTS IN THE REGULATION OF BRONCHOMOTOR TONE IN GUINEA PIGS**, submitted by **Sudhir J.A. D'Souza** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**.

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Date: February 1, 1990

To Egbert and Nita who provided the opportunity

and

To Lina who provided the encouragement

ABSTRACT

1) In guinea pigs, orthodromic electrical stimulation of carotid-sinus-nerve (CSN) afferents was reported to mimic reflex bronchoconstriction induced by acetyl salicylic acid (ASA), indomethacin (IND), or tartrazine (TZ) via sympathetic afferents. The aim of this thesis was to examine the role of CSN afferents and sympathetic efferents in the regulation of bronchomotor tone.

2) In anesthetized guinea pigs, TZ, ASA, and IND (i.v.) increased CSN activity and mean arterial blood pressure (MABP). The increases in MABP and CSN activity were not correlated, suggesting a direct action on CSN afferent endings. In a vascularly-isolated carotid sinus preparation, TZ had little effect on carotid-body chemoreceptor (CCr) activity, but increased carotid baroreceptor activity (CBr).

3) Stimulating CBr either electrically or by increasing intrasinus pressure (ISP) had no effect on bronchomotor tone, but decreased MABP and ventilation. Decreasing ISP induced bronchoconstriction and increased ventilation. Also, NaCN injected retrogradely into the sinus to stimulate CCr induced bronchoconstriction and a large increase in ventilation.

4) The effects of α-adrenoceptor stimulation on the airways of normal and ovalbumin-sensitized guinea pigs were studied. In normal animals, noradrenaline (NA) (i.v.) induced small bronchoconstriction and bronchodilation. Propranolol blocked the latter. The bronchoconstriction was dose-dependent and reproducible only in propranolol-treated animals. NA induced bronchoconstriction before and after propranolol in ovalbumin-sensitized animals. NA-induced broncho-

constriction was abolished by prazosin in both normal and ovalbumin-sensitized animals.

5) These findings indicate that CBr have no, and CCr have a minor role, in the regulation of bronchomotor tone and that α_1-adrenoceptors do not significantly affect bronchomotor tone in healthy guinea pigs.

ACKNOWLEDGEMENTS

A doctoral degree is granted to an individual on completion of a thesis, but the production of a thesis is truly a combined effort. While no one writes the thesis for you, a number of people contribute to the collection, the interpretation, and final presentation of the data. I should first like to thank the members of my committee: Drs. D.F. Biggs, R.L. Jones, C.T. Kappagoda, G.C. Man, A.S. Clanachan, and E.E. Daniels. The efforts of these gentlemen, especially those of my supervisor David Biggs and those of Dick Jones, were most appreciated. I should also like to thank the following individuals for their various contributions in the long process: Lina Dagnino, J. Brad Hunter, Gordon McCrae, Valerie Welch, and Don Whyte.

I should like to acknowledge the Alberta Heritage Foundation for Medical Research for their generous support during my three and a half year term at The University of Alberta.

Finally, I should like to thank McGill University's Faculty of Medicine for the free computer time.

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LIST OF ABBREVIATIONS

IX - Glossopharyngeal Nerve
X - Vagus Nerve
AII - Angiotensin II
AIII - Angiotensin III
ACh - Acetylcholine
ADN - Aortic Depressor Nerve
ASA - Acetylsalicylic Acid
APA - Anterior Pharyngeal Artery
C - Compliance
CB - Carotid Body
CBr - Carotid Baroreceptors
CCA - Common Carotid Artery
CCr - Carotid body Chemoreceptor
CS - Carotid Sinus
CSN - Carotid Sinus Nerve
CST - Cervical Sympathetic Trunk
E - Elastance
ECA - External Carotid Artery
f - Rate of Respiration
FRC - Functional Residual Capacity
HR - Heart Rate
I - Inertance
i.p. - Intraperitoneal
i.v. - Intravenous
ICA - Internal Carotid Artery
IND - Indomethacin
Ig - Immunoglobulin
ISP - Intrasinus Pressure
ITA - Inferior Thyroid Artery
LT - Leukotriene
MABP - Mean Arterial Blood Pressure
NA - Noradrenaline
NANC - Non-adenergic, non-cholinergic
OA - Occipital Artery

P - Pressure
PE - Phenylephrine
PVR - Peripheral Vascular Resistance
PG - Prostaglandin
R - Resistance
RAR - Rapidly-Adapting Receptors
SAR - Slowly-Adapting Receptors
s.c. - subcutaneous
SCG - Sodium Cromoglycate
sG_{aw} - Specific Airway Conductance
SP - Substance P
TZ - Tartrazine
V - Volume
$\dot{V}$ - Rate of Airflow
V - Volume Acceleration
$\dot{V}_e$ - Minute Ventilation
V_t - Tidal Volume
VIP - Vasoactive Intestinal Peptide
VP - Vasopressin

Suffixes

alv - alveolar
ao - airway opening
av - airway
B - atmosphere
es - esophageal
L - pulmonary/lung
pl - pleural
T - thoracic
tp - transpulmonary

CHAPTER I

Literature Review and Thesis Proposal

Introduction

Asthma is manifested by widespread narrowing of the airways in response to a variety of unrelated stimuli (125, 157, 168, 189), and is characterized by bronchoconstriction, mucosal edema, and increased mucus secretion (168). Most asthmatics are hyperresponsive to many stimuli including pharmacologic and allergenic aerosols, chemical and physical agents, infections, exercise, and emotional stress (51, 168). Hyperresponsiveness can occur without the manifestations of clinical asthma (124, 125); however the majority of asthmatics develop it (122). At autopsy, asthmatic lungs show widespread inflammation. Many airways are occluded with viscid mucus, the airway epithelium is either disrupted or denuded, and the bronchiolar walls are infiltrated with granulocytes and mast cells. In severe cases, hypertrophy of airway smooth muscle and the basement membrane is present (121, 122).

No single etiology has been traced in asthma, thus most definitions or descriptions of the disease avoid reference to its pathogenesis (168). For simplicity the various forms of asthma have been defined according to their major stimuli. These classifications are artificial and asthmatic attacks can be induced by more than one stimulus (168). Asthma has been classified as: 1) Allergenic (extrinsic); 2) Infectious; 3) Psychological; 4) Occupational; 5) Exercise-induced; 6) Drug-induced (168, 189).

The aim of this thesis was to determine the roles of carotid-sinus (CSN) afferents and sympathetic efferents in the regulation of airway patency, and to determine the role, if any, of these neural systems in a guinea-pig model of drug-induced asthma. In this chapter, I shall outline the anatomy of the respiratory system and review the literature on the regulation of airway caliber with specific reference to the role of CSN afferents and sympathetic efferents. Finally, I shall describe drug-induced asthma and present the thesis proposal.

Anatomy and Morphology of the Respiratory System

i) Architecture

The respiratory system can be divided anatomically into the upper and lower respiratory tracts which are separated at the pharynx (part of the gastrointestinal system) (87, 269). The upper tract comprises the nose and nasopharynx which serve to filter, humidify, and warm the air. The lower tract begins at the larynx and continues through the tracheobronchial tree to the alveoli. All experiments in this thesis were carried out in tracheotomized animals; thus I will limit the discussion to the tracheobronchial tree and the alveoli, hereafter referred to as the "airways".

The largest airway is the trachea. It is a flexible tube of connective tissue and C-shaped cartilage which expands and extends in length during inspiration, and passively recoils during expiration (269). The trachea divides into the right and left bronchi which are made up of fibroelastic tissue interspersed with plates of cartilage. The bronchi subdivide irregularly decreasing in length and diameter, but increasing in total surface area with every generation. Bronchi give rise to bronchioles, which in humans are defined as small airways, less than 2 mm in diameter, made up of fibroelastic connective tissue devoid of cartilage (56, 87). The airways from the trachea (generation

0) to the terminal bronchioles (generation 16) are classified as conducting airways (56).

Terminal bronchioles divide into many respiratory bronchioles which, in turn, open up into alveolar ducts. Respiratory bronchioles are thinner and shorter than the terminal bronchioles. Alveolar ducts are frequently interrupted by alveolar outpouchings and terminate blindly in alveoli. Respiratory bronchioles and alveolar ducts are a transitional zone (56, 87). They conduct air and are involved in gas exchange. Alveoli are responsible only for gas exchange (56).

ii) Smooth Muscle

Smooth muscle about the bronchi and the bronchioles, including the respiratory bronchioles, is disposed in a geodesic fashion, such that contraction reduces the length and the diameter of the tubes. The fibroelastic connective tissue and the smooth muscle of the ducts are organized in rings around the opening to the alveolar sacs and alveoli. These rings serve to regulate the entry of air into the alveoli (269). Alveoli do not contain smooth muscle, but are supported by a thin layer of fibroelastic tissue.

Changes in smooth muscle tone affect pulmonary function. Contraction of the large conducting airways is reflected as an increase in resistance (170). By contrast, increases in smooth muscle tone of small-conducting and transitional airways are reflected in a change in resistance and compliance (14).

Contraction of airway smooth muscle appears to play an important role in asthma when airway obstruction is easily and rapidly reversed by bronchodilator therapy. Recently, more consideration has been given to the underlying changes in smooth muscle in asthma (240, 241). Briefly, these include:

1) Conversion of multiunit airway smooth muscle to single-unit: Multiunit smooth muscle is characterized by tonic mechanical properties, low-impedance gap junctions, and discrete neural innervation. Single-unit smooth muscle is mechanically phasic, has a high number of low-impedance gap junctions, limited innervation, and functions as a syncytium. Canine trachea has many gap junctions and is poorly innervated, but is multiunit (241). The multiunit nature of this muscle is apparently due to rectifying K^+ channels (141). Tetraethylammonium chloride can induce canine tracheal smooth muscle to behave as a single unit (141, 240, 241). A similar transformation is believed to occur to a limited extent in the airways of a canine model of asthma (240). Guinea-pig airways are more densely innervated than canine airways (212). This suggests that guinea-pig airway smooth muscle has fewer gap junctions and behaves more like a multiunit smooth muscle. It is unknown at this time if guinea-pig airway smooth muscle can also be induced to act as a single unit.

2) Changes in the mechanical properties of airway smooth muscle: Exaggerated bronchoconstriction can be induced in asthmatic airways by a variety of stimuli suggesting that hyperresponsiveness of asthmatic airways is due to a change in the muscle itself. Mechanical studies show increased maximal velocity of contraction in sensitized airway smooth muscle (240, 241).

3) Changes in the number and ratio of adrenoceptors and muscarinic receptors elaborated by the muscle: Mita et al. (171) claim that the number of muscarinic receptors is increased in sensitized guinea pigs. The number of α- and β-adrenoceptors is also thought to change in asthma (245). These changes will be discussed in more detail in the section on the autonomic system.

iii) Airway Epithelium

The airways are lined by an uninterrupted epithelium which acts as a barrier between the interstitium and the air spaces (264, 265). Airway epithelium consists of lining and secretory cells. The lining cells of the conducting airways are ciliated. Interspersed between the ciliated cells are the mucous and serous secretory cells. Mucous (goblet) cells are common in large human airways, but are rare in the airways of small animals such as rats and mice (264). Secretory cells are also found associated in small submucosal glands connected via short ducts to the lumen of the trachea and bronchi (179).

The product of the secretory cells is disposed as a thin, sticky film on the luminal surface. This film serves to trap small airborne particulate matter and debris and is continually propelled by the ciliated cells towards the larynx and the pharynx, where it is either swallowed or coughed out (264). Abnormal secretion and ciliary dysfunction can cause this thin lining to thicken and increase in viscosity, resulting in a decrease in airway caliber (179).

The epithelium gradually changes to a simple cuboidal appearance in the bronchioles. The number of secretory cells also decreases from the trachea to the terminal bronchioles (269). Respiratory bronchioles are devoid of mucous cells, and are lined by ciliated cuboidal cells and non-ciliated Clara cells. The alveoli are lined by squamous Type I pneumocytes, which constitute part of the gas diffusion barrier. A second rounded cell, the Type II pneumocyte, is also found. This cell secretes surfactant, a substance which reduces surface tension and helps prevent atelectasis during expiration (87, 265, 269). Epithelial cells express relaxing factor(s) which may contribute to the regulation of bronchomotor tone. Damage to respiratory epithelium may contribute to bronchial hyperresponsiveness by reduced release of this epithelium-derived relaxing factor(s) (258).

iv) Blood Supply

The airways are supplied by two arterial systems. The major supply is via the pulmonary system, which carries deoxygenated blood from the right ventricle to the gas-exchanging airways. The other, the bronchial system, arises from the thoracic aorta and supplies the conducting airways. The gas-exchanging airways are drained by the pulmonary veins; the conducting airways drain into both the bronchial and pulmonary venous system (87).

There is growing evidence that the bronchial system may play a role in the pathophysiology of hyperreactivity (18). Particularly because it appears to be involved in the transport of mediators and in the development of airway wall edema.

Pulmonary Defense System

Although the mucociliary system is efficient at protecting the conducting airways by trapping inhaled particulate matter, many particles containing pathogens reach the airways. The airways maintain a highly-developed defense system to combat any potential hazards.

The non-specific response in this system is mediated by macrophages. Pulmonary macrophages are divided into two groups: alveolar and interstitial. Macrophages residing in the airspaces are responsible for the clearance of particulate matter from the alveoli (87). A minority of macrophages secrete mediators and numerous enzymes important in the initiation and regulation of inflammation (204).

The specific response is mediated by lymphocytes and plasma cells organized into lymph-node-like groups along the large conducting airways. This so-called BALT (bronchus-associated-lymphoid-tissue) is similar to the Peyer's patches found in the gut and is an important component of the body's immune system (40, 87).

Granulocytes, such as eosinophils, neutrophils, basophils, and mast cells, are regularly found with the other free cells in the bronchial walls (265). These cells migrate into tissues and traverse the endothelium under the influence of chemotactic factors released by macrophages. Neutrophils accumulate during inflammation. Eosinophils are found in increased numbers in infections and allergic diseases, including asthma (116).

Mast cells and basophils are abundant in the trachea, the pleura, in the connective tissue of the bronchi and bronchioles, and in the alveolar septa (94, 116, 264). Mast cells are a major source of the chemical mediators that are known to play a role in allergic inflammatory reactions such as airway anaphylaxis and asthma (80). However, they are not the only source; eosinophils, basophils, and neutrophils also contribute (94, 116).

The classic mast-cell reaction is triggered by allergens that bridge specific immunoglobulin (Ig) E antibodies on the cell surface to induce the discharge of chemical mediators. Other stimuli are capable of initiating mast-cell degranulation, including: hypoxia, acetylcholine, peptides, such as the opioids, and physical stimuli such as cold (94).

Chemical mediators released by mast cells can be divided into two categories: 1) preformed or granule-associated mediators, and 2) newly-generated mediators (80, 94). Preformed mediators include histamine and 5-hydroxytryptamine. Histamine is the oldest known mediator of hypersensitivity. It has both direct and indirect effects on human and guinea-pig airways (41, 80, 270). The actions of histamine are mediated by two types of receptors designated H_1 and H_2. H_1-agonists increase smooth-muscle contraction, vascular permeability, and activation of vagal afferent nerves. H_2-agonists induce bronchodilation, airway mucus secretion (humans only) and inhibit histamine release from basophils.

5-Hydroxytryptamine (5-HT) is a potent constrictor of guinea-pig airways, but has limited effects in humans (80).

Newly-formed mediators are mainly arachidonic acid metabolites. Prostaglandin (PG) are derived via the cyclooxygenase pathway. Prostaglandin G_2 and PGH_2, and their metabolite PGD_2 constrict guinea-pig airways, and PGE_1, PGE_2, and PGI_2 relax them (80). Slow-reacting substance of anaphylaxis (SRS-A) is the main product of the 5-lipoxygenase pathway. It is composed of leukotriene (LT) C_4, LTD_4, and LTE_4, the most potent constrictors of human and guinea-pig airway smooth muscle known to date (80, 94, 116). Although allergic or extrinsic asthma is but one form of this disease, aberrant formation of IgE against innocuous antigens leading to release of mast cell mediators has often been suggested as the underlying "fault" leading to the disease (see 1, 20, 140). The similarities between asthma and anaphylaxis have been used to develop experimental-animal models of the disease (234). A popular model is the ovalbumin-sensitized guinea pig. The development and use of this model in the investigation of asthma has been recently reviewed (140). Although variations on this model are common (25), the basic protocol remains as described by Pavel and Kallos in 1938 (see 140). Briefly, guinea pigs are given ovalbumin i.p. and, after 2-3 weeks, are exposed repeatedly to an ovalbumin aerosol. Exposure to the aerosol induces acute respiratory distress and can lead to death. Animals are sensitive to this challenge for about 12 months after sensitization.

There is currently a debate as to the nature of the antibody involved. A so-called short-term IgG_1 antibody is believed to be induced in animals sensitized with ovalbumin only, and a combination of IgE and IgG_1 in animals sensitized with ovalbumin accompanied by adjuvants such as alum, Ascaris, or Freund's complete adjuvant (140). Ovalbumin sensitization with aluminum hydroxide adjuvant is reported to induce IgE production only (11).

Ovalbumin-sensitized guinea pigs, like asthmatics, show an exaggerated bronchoconstriction to chemical and mechanical stimuli that is localized in the small airways (81).

Autonomic Regulation of the Airways

Autonomic innervation to the airways is composed of the parasympathetic, sympathetic, and a third, so-called NANC (non-adrenergic and non-cholinergic) system. All three systems are involved in the regulation of smooth-muscle tone and airway patency. My work has focussed on the sympathetic system and its role in regulating airway smooth muscle. In this section, I intend to outline briefly the role of the parasympathetic and NANC innervations, and subsequently review the sympathetic innervation in greater depth.

i) Parasympathetic innervation to the airways

Parasympathetic nerve supply to the airways is via the vagus nerves. Preganglionic fibers travel from the brainstem to the airway ganglia, and postganglionic fibers from these ganglia extend to the airway smooth muscle and submucosal glands (212). Both pre- and postganglionic fibers release acetylcholine (ACh) which acts on nicotinic receptors at the ganglia and on muscarinic receptors at the target organs.

The parasympathetic is the dominant neural bronchoconstrictor system and is responsible for maintaining airway tone (22, 24, 52, 70, 178, 180, 181). The presence of vagal tone can be demonstrated by cutting or cooling the vagi, or by giving muscarinic antagonists. Electrical stimulation of the vagus nerves induces bronchoconstriction which is enhanced by acetylcholinesterase inhibitors and blocked by atropine. The density of the muscarinic receptors and the distribution of cholinergic innervation is highest in the large conducting airways and sparse or absent in the small airways of most animals (24, 149), reflecting the relative contributions of the large conducting and small airways to vagally-mediated bronchoconstriction.

The parasympathetic system also affects airway diameter by increasing secretions from the submucosal glands (179). Cholinergic agonists have been reported to enhance the release of mast cell mediators in human lung *in vitro* (139, 176), but not *in vivo* (127). Some mast-cell mediators such as histamine and 5-HT can initiate the release of ACh from postganglionic nerve terminals, whereas other agents such as PGE_2 have the opposite effect (22, 24, 52, 136). Cholinergic activity may also be modulated by the actions of β- and α- adrenoceptor agonists on neurotransmission through the ganglia. Ganglionic transmission may also be influenced by various inflammatory mediators (22). Acetylcholine acting on prejunctional inhibitory muscarinic receptors is a potent inhibitor of its own release (160).

Imbalance or hyperreactivity of the parasympathetic system has been implicated as a possible cause of asthma. Most asthmatics demonstrate an exaggerated bronchoconstrictor response to muscarinic agonists, and muscarinic antagonists are effective bronchodilators. The parasympathetic system should not be considered as solely a motor system. Although there is no direct evidence for a hyperresponsive parasympathetic system or increased parasympathetic tone as the primary cause of asthma (22, 24, 51, 52, 180, 181, 273), the afferent innervation which can influence cholinergic activity reflexly must be considered. Aberrant or hyperresponsive afferent pathways can also lead to, or induce, abnormal parasympathetic responses (181).

### ii) Nonadrenergic and noncholinergic innervation (NANC)

The NANC system is composed of an inhibitory nonadrenergic and an excitatory noncholinergic innervation. The nonadrenergic system is considered to be the main inhibitory pathway in human airways (22, 52, 149) and has been demonstrated in the airways of several species (212), including guinea pigs (61, 76). The system was originally thought to be purinergic, however current reports suggest that the neurotransmitter is vasoactive intestinal peptide (VIP) (22, 158, 196, 215). VIP-like immunoreactivity in nerve profiles has been demonstrated adjacent to smooth muscle in the large conducting airways, but not in the small airways (149, 150, 159). Although VIP meets most of the criteria for a neurotransmitter in the airways (see 22, 215), its weak effects in human airway tissue and a residual neurally-mediated inhibitory component following VIP desensitization suggest that it is not the only neurotransmitter involved in nonadrenergic inhibition of the airways (22, 158).

A noncholinergic excitatory pathway has been demonstrated in guinea-pig airways. The mediator for this noncholinergic system is believed to be substance P (SP) (149, 158, 196). Electrical stimulation of the vagi after muscarinic blockade induces a bronchospasm which is inhibited by SP antagonists, but unaltered by ganglionic blockers (158). This led to the suggestion that the noncholinergic bronchospasm is due to the release of SP by antidromic stimulation of vagal afferents. It has been further suggested that the release of SP and other sensory peptides affecting smooth muscle can be induced by local axonal reflexes selectively stimulated by chemical mediators or by local injury (22).

iii) Sympathetic nervous system in the airways

Sympathetic innervation to the airways originates from the upper six thoracic segments. The cholinergic preganglionic sympathetic efferents synapse in cervical and thoracic sympathetic ganglia via nicotinic receptors. The noradrenergic postganglionic fibers enter the lung and intertwine with fibers of the parasympathetic system. Postganglionic sympathetic nerves act on β- and α-adrenoceptors on the target organs.

By contrast to the cholinergic innervation, the sympathetic innervation of the airways is sparse in most animals (212). It is generally accepted that the bulk of airway sympathetic innervation is to the submucosal glands and the smooth muscle of the pulmonary vasculature (149, 151, 227). Some recent ultrastructural studies on human bronchi obtained at biopsy have shown a significant number of putative adrenergic nerves supplying bronchial smooth muscle (69, 193). These nerves are believed to be adrenergic because they contain among other vesicles, small granular vesicles (69) which may be labelled with 5-hydroxydopamine (193). Similar nerves have also been found in ultrastructural studies of canine bronchi (E.E. Daniel, personal communication). Equivalent evidence in favor of an adrenergic innervation in guinea pigs is not available. At present, in guinea pigs, sympathetic fibers have been found concentrated in cervical-tracheal smooth muscle, but limited to vascular smooth muscle with the occasional fiber in the vicinity of the smooth muscle in the thoracic trachea and in the bronchi (128, 137, 190, 191, 233).

The distribution of the sympathetic innervation has been confirmed by functional studies. Neurally-mediated relaxation of human airways is unaffected by propranolol (250), whereas neurally-mediated relaxation of guinea-pig trachea, but not bronchi, is blocked by β-adrenoceptors antagonists (76, 277, 278). Uptake mechanisms for noradrenaline (NA) are also present in guinea-pig trachea (90). Blockers of neuronal and extraneuronal uptake of NA potentiate the response to NA in guinea-pig trachea, but not in bronchi (277, 278). This suggests that guinea-pig trachea has functional noradrenergic innervation, whereas guinea-pig bronchi and human airways do not. However, because vascular and airway smooth muscle are adjacent, NA released in the vasculature could act on airway smooth muscle. Blockade of the neuronal and extraneuronal uptake of NA should facilitate the diffusion of NA out of vascular smooth muscle. It is of interest that electrical stimulation of sympathetic _in vivo_ outflow induces a bronchodilation (8) which has been ascribed to the overflow of NA from the well-innervated vascular smooth muscle (8, 76).

There is some evidence that sympathetic innervation can modify cholinergic transmission. Sympathetic fibers have been localized in parasympathetic ganglia in human airways (149). Noradrenaline inhibits ganglionic neurotransmission via α-agonists (19). Sympathetic stimulation inhibits neurotransmission at parasympathetic ganglia and prejunctional terminals (19, 106, 107, 259). This may explain why vagal bronchomotor tone must be present to demonstrate the effect of sympathetic nerve stimulation (178). However, Martin and Collier (164) were unable to detect any change in the amount of ACh released in response to electrical field stimulation of canine tracheal smooth muscle in the presence and the absence of NA.

Human-airway smooth muscle is relaxed by adrenergic agonists acting on β-adrenoceptors. While sympathetic innervation decreases as the size of the airways decreases, the density of the adrenoceptors on airway smooth muscle increases (22, 23, 277). The absence of functional innervation suggests that these receptors are stimulated by circulating catecholamines secreted by the adrenal medulla. The evidence indicates that the dominant circulating catecholamine acting on airway smooth muscle is adrenaline which is a potent bronchodilator in normals and asthmatics (27, 262). By contrast, high levels of circulating NA have little effect on airway smooth muscle (73, 152).

There are two types of β-adrenoceptors - β_1 and β_2. The latter dominate in the airways of man and most animals (58, 277). The relative distribution of β_1- and β_2-adrenoceptors reflects their relative roles in the airways (58). β_1-adrenoceptors are associated primarily with the sympathetic innervation in the large conducting airways, whereas β_2-adrenoceptors are found in the smaller airways. In humans, only β_2-adrenoceptors mediate the relaxation induced by sympathomimetic amines (99), even though both β_1- and β_2-adrenoceptors are found in the airways (58). In guinea pigs, β_1- and β_2-adrenoceptors are found throughout the airways and both are involved in the relaxation of tracheal smooth muscle, but only β_2 mediate the relaxation of the smaller airways (278).

β-adrenoceptors are believed to mediate prejunctional inhibition of cholinergic transmission (259). β-adrenoceptor agonists also inhibit the release of mast-cell mediators (139, 176) and promote the secretion of mucus in several species (179).

β-adrenoceptors have little role in maintaining airway patency at rest, but they appear to have a protective role in hyperresponsive airways of asthmatics. Also, they may dampen airway response to challenges and mediate recovery (277). β-antagonists do not affect bronchomotor tone in normal subjects, but can induce bronchoconstriction in asthmatics and potentiate and prolong airway responses to noxious stimuli, mast cell mediators, parasympathetic stimulation, and exogenous ACh (22, 72, 79, 167, 273). The lack of a functional innervation suggests that the increased β-adrenoceptor tone in asthmatics is mediated by circulating adrenaline.

The role of β-adrenoceptors in animals is controversial. Propranolol induces bronchoconstriction in dogs, which suggests that canine airways are under tonic β-adrenoceptor control (268). Similar responses have been reported in guinea pigs (3, 161, 166, 186), but others have been unable to confirm these findings (59). The propranolol-induced bronchoconstriction is apparently a non-specific effect, as both l- and d-propranolol are equally potent in inducing bronchoconstriction (161, 186).

Szentivanyi (245) proposed that a general defect in β-adrenoceptor numbers or responsiveness could account for asthma. There is mixed evidence in favor of this hypothesis. Several studies have shown impaired β-adrenoceptor sensitivity in leukocytes derived from asthmatics, but other studies have claimed normal β-adrenoceptor function (see 152). The ratio of β- to α-adrenoceptors in asthmatic lungs has been reported to be lower than that in normal lungs (246). Similar changes have been reported in ovalbumin-sensitized guinea-pigs (25, 172). The decrease in β-adrenoceptor binding in ovalbumin-sensitized guinea pigs is limited to the alveolar and conducting airway epithelium, and bronchiolar and vascular smooth muscle (95).

Functional studies of airway smooth muscle have been inconclusive. Studies on asthmatics found reduced plasma levels of cyclic adenosine monophosphate (cAMP) in response to infusion of β-agonists (i.v.) or to administration of NA (s.c.) (15, 225), but airway responses to β-agonists were similar in asthmatics and healthy controls (249). Sand (217) reported a decrease in the basal cAMP levels in ovalbumin-sensitized guinea-pig airways; but, others have reported that β-adrenoceptor-mediated responses were unaltered by ovalbumin-sensitization (165, 187). Souhrada et al. (237) found reduced β-adrenoceptor and purine-mediated relaxation in guinea pigs sensitized to ovalbumin with pertussis-vaccine adjuvant. Pertussis vaccination is claimed to reduce β-adrenoceptor binding in guinea-pig airways (171).

Whether β-adrenoceptor function is impaired in asthmatics is unresolved. Barnes (21, 23) states that such a defect is of little clinical significance since asthmatics bronchodilate readily to β-agonists. However, Larsson (152) has shown that although the decrease in β-adrenoceptor number and function is not critical in all asthmatics, it may be clinically important in exercise-induced asthma. Others have argued that the reduction in β-adrenoceptor numbers seen in various animal models of asthma may not be a primary change, but represents "down regulation" to compensate for increased sympathetic tone seen in these models, and in asthmatics (221).

α-adrenoceptors have also been localized in the airways, but they are scarce. The ratio of β- to α-adrenoceptors is 6:1 in normal human airways (246), and 16:1 in normal guinea-pig airways (30). The density of α-adrenoceptors, like that of β-adrenoceptors, increases as the airways decrease in size (30).

Studies _in vivo_ and _in vitro_ have shown α-adrenoceptor-mediated bronchoconstriction in man (47, 48, 230, 235), dogs (31, 146, 154), and guinea pigs (5, 148). α-agonists may induce bronchoconstriction by direct actions or by facilitating the release of mast-cell mediators (139). They can also affect airway patency by increasing serous secretions from submucosal glands (179).

Both α_1- and α_2-adrenoceptors are found in the airways (106, 107, 154). In canine trachea, the contractile response is mediated through α_1- and α_2-adrenoceptors (154), but the post-junctional α_2-adrenoceptors dominate (31). Humans and guinea pigs appear not to have post-junctional α_2-adrenoceptors (13). α-agonist-induced bronchoconstriction is mediated via α_1-adrenoceptors in humans and guinea pigs (230, 277). Stimulation of prejunctional α_2-adrenoceptors has been shown to inhibit cholinergic transmission in human airways (106), and cholinergic (107) and NANC excitatory transmission in guinea pigs (108). Phentolamine, an α-antagonist, has also been shown to block NA-induced inhibition of parasympathetic ganglionic activity in rats and ferrets (19).

The role of α-adrenoceptors in regulation of airway smooth muscle is still controversial (13, 100). Studies have shown that α-agonists have little effect in normal human (100, 198, 230), and canine airways (31, 154), even in the presence of β-blockade. However, they can induce bronchoconstriction in canine trachea and bronchi incubated with histamine, 5-HT, and potassium (31, 146), and in sensitized human airways (230). It has been suggested that mast-cell mediators enhance α-adrenoceptor function in airway smooth muscle. However, others report that in vitro α-agonists contract canine tracheal and bronchial smooth muscle in the absence of β-blockade (33). Black et al. (40) claim that normal human peripheral lung strips contract to NA, but suggest that this response may be due to α-adrenoceptors on contractile elements such as pulmonary vessels.

α-antagonists such as phentolamine and thymoxamine are claimed to inhibit bronchoconstriction induced by histamine (143), allergen (198), and exercise (39, 260). These antagonists display antihistaminic activity, influence catecholamine uptake and release, and have direct actions of their own on airway smooth muscle (22, 37, 100, 152). By contrast, prazosin, a selective α_1-adrenoceptor antagonist, does not affect airway function in asthmatics, nor does it affect histamine-induced bronchoconstriction in young asthmatics (29). Prazosin is effective against exercise-induced bronchoconstriction (29) and histamine-induced bronchoconstriction in elderly asthmatics (133).

The role of α-adrenoceptors in regulation of airway patency in guinea pigs is also controversial. In vitro, after β-blockade, α_1-agonists contract guinea-pig trachea and bronchus, but, in vivo, have little effect and in fact reduce the bronchoconstriction induced by histamine, 5-HT, and ACh (5). Kreutner and Rizzo (148) claim that NA in the presence of propranolol induces bronchoconstriction in normal guinea pigs. Noradrenaline, at the doses used in their study, has a powerful effect on the vasculature. Similar doses of NA induce bronchoconstriction in rats, and it has been suggested that this bronchoconstriction and that described in guinea pigs is induced secondarily to changes in the vasculature (73).

Szentivanyi (245) suggested that bronchial hyperreactivity in asthma may be associated with increased α-adrenoceptor stimulation coupled with diminished β-adrenergic function. The evidence in favor of this theory is still weak. Binding studies have shown that α-adrenoceptors increase in asthmatics (246), and in ovalbumin-sensitized guinea pigs (25, 172). Functional studies have not been as clear. In some studies, inhaled α_1-agonists induced weak bronchoconstriction in asthmatics (47, 230, 235), but others were unable to confirm these findings, even in the presence of a β-blocker (251). Kneussl and Richardson (146) described α-adrenoceptor hyperresponsiveness in bronchial smooth muscle derived at autopsy from patients with asthma and other forms of pulmonary disease. However, Goldie et al. (100) showed that α-adrenoceptor activity is not enhanced in isolated bronchial smooth muscle from asthmatics. Moreover, in guinea pigs, ovalbumin sensitization had little effect on NA-induced contractions of peripheral lung strips (256). Goldie and his colleagues suggested that the development and exacerbation of asthma is not dependent on increased α-adrenoceptor activity and that these receptors have no role in the regulation of airway patency. This view has been criticized by Andersson et al. (13), who contend that prejunctional α_2-adrenoceptors have a role maintaining airway patency in diseased and sensitized airways.

Recently, Andersson's group showed that clonidine, at very low doses, reduces allergen-induced bronchoconstriction in asthmatics (155, 156), and ovalbumin-sensitized guinea pigs (12). However, Advenier et al. (6) claim that in normal guinea pigs clonidine potentiates the bronchoconstrictor effects of histamine, 5-HT, and ACh. This has been confirmed by Andersson's group (155). Advenier and his colleagues attempted to explain their finding by pointing out that α_2-adrenoceptors also impair NA release. However, as I stated earlier, the evidence in favor of a functional noradrenergic innervation to guinea pig airways is scanty. Thus, this explanation seems implausible.

Afferent Nerve Supply to the Airways

The afferent nerve supply to the airways has been the subject of thorough reviews recently (62, 218). Pulmonary afferents may be divided into vagal and sympathetic. Sympathetic afferent activity with a clear respiratory rhythm has been localized in the upper thoracic white rami communicantes of dogs. They appear to mediate the excitatory effects of certain chemical and mechanical stimuli.

The majority of pulmonary afferents travel in the vagus. Vagal afferents consist of myelinated fibers innervating slowly-adapting (SAR) and the rapidly-adapting (RAR) stretch receptors, and unmyelinated C-fibers. SAR-activity increases with inspiration and provides an off-switch for inspiration. Stimulation of these SAR induces apnea (Hering-Breuer reflex), bronchodilation, tachycardia, and vasodilation.

Rapidly-adapting receptors respond to large inflations and deflations, but show irregular and scanty activity in eupnea. They are also activated by chemical irritants and bronchoconstrictors, leading some researchers to call them "irritant" receptors (194).

C-fiber afferents are divided physiologically and pharmacologically into pulmonary and bronchial C-fibers, reflecting their blood supply. Stimulation of these afferents induces apnea followed by rapid shallow breathing, cough, bronchoconstriction, and increased mucus secretion. Both types of C-fiber endings respond to chemical and mechanical stimulation. Bronchial C-fibers are more sensitive to chemical stimulation than the pulmonary, which are more sensitive to mechanical stimulation (218). Pulmonary C-afferents are thought to be sensors for pulmonary congestion and edema (194).

Cardiovascular receptors in the regulation of the airways

In addition to the pulmonary receptors, other receptors located in the heart and the systemic circulation are involved in the regulation of airway function. These receptors include: 1) Cardiopulmonary receptors (67); 2) Pulmonary vascular receptors in the pulmonary artery and its major branches (104, 105); 3) Arterial baroreceptors (67, 214); 4) Arterial chemoreceptors (86). The roles of these afferents in the regulation of the airways and the interactions between the respiratory and circulatory systems have been reviewed recently (67). Only the latter two receptors and their roles in the regulation of airway function will be discussed, as they are the subject of this thesis.

Arterial baroreceptors are located in the aortic arch, brachiocephalic artery, and the bifurcation of the common carotid artery (CCA) (17, 145). They are slowly-adapting mechanoreceptors which respond to mechanical strain of the arterial wall secondary to a rise in blood pressure (17, 53, 145, 214). If the arterial wall is prevented from stretching in response to an elevation in pressure, baroreceptors are not activated (53). Baroreceptors also discharge when the shape of the sinus wall is distorted by a large fall in pressure (92).

Chemoreceptors are located in the ascending aorta, aortic arch, and in the carotid body, which is found at the bifurcation of the CCA. They respond to changes in the arterial blood PO_2, PCO_2, and pH (86).

Baroreceptor fibers from the aorta run either separately as the aortic depressor nerve (ADN) in guinea pigs (242) and in rabbits, or in the vagus in dogs (53). Chemoreceptor fibers from the aorta run in the vagus. The CSN contains carotid-body chemoreceptor (CCr) and carotid baroreceptor (CBr) afferents, and efferent fibers to the carotid body (86, 145). The CSN and ADN are readily identified electrophysiologically in the intact animal by their characteristic bursts of electrical activity in synchrony with systole.

My work has focussed on CSN afferents. In this section, I propose to review the basic characteristics of carotid baro- and chemoreceptors with specific reference to their role in respiration. I shall discuss how they interact and delineate their potential role in airway dysfunction.

i) Carotid baroreceptors

The carotid sinus is an anatomical dilatation formed at the base of the internal carotid artery (ICA) in man and most species (17). In guinea pigs, the ICA is absent and the sinus is located at the origin of the occipital artery (OA) (17, 117, 206). The sinus is highly elastic and contains less vascular smooth muscle than adjacent portions of the CCA, external carotid artery (ECA), ICA, and OA. It is well innervated. Myelinated and unmyelinated baroreceptor fibers have been located in the adventitia or extending to the medio-adventitial border in most species (17), and to the innermost layers of the media adjacent to the intima in guinea pigs (49, 228). The sinus is also innervated by efferent sympathetic fibers originating from the superior cervical ganglion (208, 228).

Baroreceptors in the aorta and the carotid sinus play a major role in the regulation of blood pressure. Sectioning the ADN and the CSN leads to acute hypertension. Vasoconstrictor agents, such as adrenaline, increase baroreceptor discharge. This increase in baroreceptor activity leads to reflex decrease in sympathetic outflow resulting in decreased peripheral vascular resistance and bradycardia (67, 214). Carotid baroreceptors appear to dominate over aortic baroreceptors in most species (17). Decreasing intrasinus pressure (ISP) or increasing aortic pressure by bilateral occlusion of the CCA produces hypertension even with the ADN intact (145). In guinea pigs, bilateral carotid occlusion results in a fall in blood pressure which is unaffected by sectioning the CSN and the ADN, but which is blocked by bilateral vagotomy (42).

Baroreceptor stimulation decreases minute ventilation ($\dot{V}_e$) in most animals (67). Large increases in blood pressure induced by agents such as adrenaline cause a marked reduction in $\dot{V}_e$ and, occasionally, apnea

(134). Distending the aorta or increasing ISP results in a decrease in $\dot{V}_e$ (50, 54, 109). The decrease in $\dot{V}_e$ is due primarily to slowed breathing, as tidal volume (V_t) remains either constant or is elevated after CBr stimulation. Carotid baroreceptor stimulation alters time of inspiration (T_i) (238). Increasing arterial blood pressure while ISP was held constant, had no effect on respiration. Decreasing ISP had the opposite effect on $\dot{V}_e$, respiratory rate (f), and V_t (54, 138).

Several methods have been used to study the response characteristics of CBr and the baroreflex. Most data have come from studies using isolated, perfused carotid-sinus preparations to study the relationship among intrasinus pressure (ISP) and CBr discharge and reflex changes in circulation or respiration. This preparation allows for independent and fine control of ISP and isolates the input from potential changes in arterial blood pressure. The best preparation is obtained in the dog, in which the carotid body is easily isolated surgically from the carotid bifurcation (54). In animals such as cats (50, 96) and rabbits (92) this is not possible. Some researchers have tried to exclude CCr in these animals by inducing thrombosis in the small vessels supplying the carotid body (92) or by desensitizing the receptors by injecting acetic acid or lobeline (96). Others have opted to control chemoreceptor activity by perfusing the sinus with the animal's own blood or to cross-perfuse it with that of another, after ensuring that the animals were well ventilated and that the blood-gas values were stable (50, 117).

A static, non-pulsatile stepwise increase in ISP causes an increase in CBr activity. The change in CBr activity has two components: 1) a dynamic, rapidly adapting (< 5 s), and 2) a static, steady-state discharge. Carotid-baroreceptor activity is not the only variable used to evaluate CBr-response to incremental changes in ISP. Reflex changes in blood pressure and sympathetic outflow are also commonly used. The curves relating blood pressure, sympathetic outflow, and CBr activity to ISP are similar (92). This reflects the close correspondence between CBr activity and the reflex effects of CBr stimulation.

Curves relating steady-state-CBr discharge and blood pressure to ISP vary among species. However, in general, one can say that baroreceptors only discharge when ISP is raised to a threshold value and thereafter the firing rate increases in linear fashion with ISP until a saturation value is reached. The slope of the linear portion of this curve is used as a measure of CBr sensitivity (214).

Between the threshold and saturation pressures when ISP is increased and decreased, the curves relating ISP to CSN activity show hysteresis whether the input pressure is pulsatile or non-pulsatile (200, 214). Similar hysteresis has been demonstrated between wall tension and aortic baroreceptor activity (64), and is also apparent when arterial blood pressure is used as the index of CBr activity (54, 138). Hysteresis may or may not be apparent when the respiration is used as an index of CBr activity because the ventilatory response is sensitive to the level of anesthesia (54, 138).

Hysteresis is held to be, in part, a manifestation of the viscoelastic properties of CBr (35, 64, 214). Baroreceptors are believed to be coupled mechanically to viscoelastic elements (elastin, collagen, smooth muscle) in the arterial wall (53). Pressure produces a circumferential strain deforming the receptors and causing them to fire. Like all viscoelastic materials, the blood vessel slowly expands after its initial response to a pressure increase (93). This late response is known as "creep". When the pressure is decreased, only a part of the initial displacement will be recovered due to creep or to the adaptive deformation of the vessel. Hysteresis in the CBr response reflects this adaptive deformation.

The static non-pulsatile threshold pressures for myelinated CBr in dogs (111, 112, 113), cats (216), rabbits (254, 275), and monkeys (252) range from 50 to 80 mm Hg. However, thresholds as low as 25 mm Hg in rabbit (17) and as high as 104 mm Hg in dog (232) have been reported. Saturation pressure ranges from 160 to 200 mm Hg for most species. Although the slope of the linear portion of the CBr activity-ISP curve is used to determine sensitivity, it is difficult to compare slopes reported among different studies, as different measures of CBr activity have been employed. However, the slopes do offer a general index of sensitivity. Threshold and saturation points and CBr sensitivity are altered when static, pulsatile changes in ISP are used to stimulate CBr (17). In general, unmyelinated fibers have a higher threshold than myelinated fibers, a lower gain, and show more rapid adaptation (275).

Because CBr respond to strain in the sinus wall, factors which alter the relationship between ISP and sinus-wall mechanics affect CBr sensitivity. Vasoconstrictors decrease the diameter of vessels and the sinus is no exception (34). Decreasing the diameter of the sinus should reduce the strain in the sinus wall and thus unload CBr. Angiotensin II, a potent vasoconstrictor, is believed to act in this fashion to attenuate CBr discharge. By contrast, other vasoconstrictors increase CBr activity. Sympathetic stimulation, exogenous NA, and vasopressin (VP) - also powerful vasoconstrictors - increase CBr activity (126, 220, 253, 276). It has been suggested that the increases in CBr activity induced by sympathomimetics, exogenous NA, and VP are due to their actions on smooth muscle. Others, however, have argued that the excitatory effects of these agents are independent of their actions on vascular smooth muscle. There is mixed evidence in favor of both hypotheses:

1) Tomomatsu and Nishi (253) demonstrated in rabbits that excitatory effects of sympathetic stimulation were not blocked by phentolamine, which suggests that these effects were not due to an increase in vascular smooth muscle tone. Furthermore, they showed that while NA (10^{-10} M) increased CBr activity, NA (10^{-7} M) decreased it. They proposed that the smooth muscle contraction occurring at higher concentrations of NA unloads CBr and that the excitatory effects of NA at the lower concentration is due to direct actions on the CBr. A similar mechanism of action has been postulated for the effects of nifedipine on CBr activity (111).

2) In rabbits, Holmes and Ledsome (126) reported that NA (10^{-6} M) excited and inhibited CBr. They proposed that CBr responses to vasoconstrictors reflect the nature of the CBr-smooth muscle coupling. Carotid baroreceptors coupled in series with smooth muscle will be excited by vasoconstrictors, whereas those coupled in parallel will be inhibited. Furthermore, they reported that VP also increases activity in some CBr while attenuating the activity of

others. They have also suggested that the similarity between NA and VP responses demonstrates that both act via smooth muscle rather than direct sensitization. This narrow interpretation assumes that VP acts solely on vascular smooth muscle. In rabbits, VP alters CBr activity and the baroreflex peripherally and centrally (220).

3) Using rabbit aortic arch, Munch et al. (177) confirmed that NA has two modes of action on baroreceptors. They showed that NA at low concentrations (10^{-10} - 10^{-7} M) inhibited CBr activity by contracting the aorta and reducing circumferential strain, but, at higher concentrations (10^{-6} - 10^{-5} M) it increased baroreceptor activity by a direct action. Paradoxically, although they use the same argument as Tomomatsu and Nishi (253) to explain the different modes of action of NA, the doses they claim to inhibit and to stimulate aortic baroreceptors, are exactly opposite to those reported by Tomomatsu and Nishi who used CBr. Munch and his colleagues have also shown that NA at low concentrations increases CBr activity if the diameter of the aorta - and, thus, strain - is held constant. They suggest that these findings indicate that NA acts directly on smooth muscle to increase baroreceptor discharge via an increase in wall tension or via contraction of smooth muscle in series with the receptor.

If the same holds for the sinus, then it is possible that NA may act both directly on the receptor and indirectly on the smooth-muscle-coupling system to increase CBr activity. The latter effect would be less prominent as it should be masked by the unloading of CBr due to contraction of the vessel.

In hypertension, CBr have higher threshold pressures and reduced sensitivity to changes in ISP (63, 112, 113). The threshold pressures and sensitivity of CBr may be also altered acutely. In rat aortic arch, the acute resetting time is consistent with its viscoelastic properties (63). However, in dog carotid sinus, acute resetting involves an ionic mechanism (112, 113). Reduced CBr sensitivity has also been reported in patients with chronic obstructive pulmonary disease (199). The cause of this attenuation has been attributed in part to pulmonary hypertension and to increased cardiopulmonary activity. However, the central effects of hypoxia and hypercapnia are clearly important.

ii) Carotid chemoreceptors

The carotid body is a highly vascularized organ located near the carotid bifurcation that draws its blood supply from one or more branches of the ICA, ECA, and OA. The organ consists of islands of cells and capillaries termed glomeruli, surrounded by connective tissue capsules. Each glomerulus or glomus is described as a miniature carotid body. The body is innervated by myelinated and unmyelinated afferent fibers from the CSN and unmyelinated post-ganglionic sympathetic efferents originating in the superior cervical ganglion (85, 88).

Carotid-body chemoreceptors respond to changes in arterial blood PO_2, PCO_2, and pH. In the resting animal, CCr discharge in synchrony with respiration as they respond to minor fluctuations in arterial PO_2 (45). However, it is difficult to distinguish this low-level discharge in the CSN of a well-ventilated animal with an intact, well-perfused carotid sinus and body. Carotid chemoreceptor activity is easily distinguished from CBr discharge in hypoxic, hypercapnic, or acidotic animals by its non-pulsatile discharge (88). Carotid chemoreceptor activity can be altered by changes in ISP (46). Rapid increases or decreases in ISP induce transient and opposite changes in CCr activity. Decreasing ISP below the CBr threshold increases CCr discharge.

A number of substances are known to increase CCr activity. These include: ACh, 5-HT, neuroactive peptides, phenyldiguanide, and sodium cyanide (85, 88, 96, 144, 169, 195). Noradrenaline at low doses and sympathetic stimulation also excite CCr; but, this is believed to be a secondary effect mediated via actions on the vasculature (86, 195).

Carotid chemoreceptor stimulation results in a significant increase in ventilation, respiratory rate, and tidal volume (89). The effects of CCr stimulation on the vasculature are variable. In general, CCr stimulation evokes bradycardia or tachycardia, and hypertension via increased sympathetic outflow (86).

iii) Interaction among carotid baroreceptor and chemoreceptor reflexes

Both CBr and CCr are affected directly in situations such as hypotension or hypoxia, and indirectly when reflex effects mediated by one group of receptors interact with the effects mediated by the other (163). This interaction appears to be mutual inhibition. Carotid baroreceptor stimulation attenuates the cardiovascular and the ventilatory responses to CCr stimulation (114, 115), and CCr stimulation inhibits the effects of CBr stimulation on the vasculature (163). Similarly, the increase in sympathetic activity induced by transient

CCr stimulation is reduced by CBr activation, and prolonged CCr stimulation blocks the CBr-induced decrease in sympathetic activity (255). Orthodromic electrical stimulation of the CSN results in increased ventilation, hypotension, and bradycardia (78). This suggests (predictably) that the reflex effects of CCr stimulation dominate on respiration, and the CBr reflex dominates on circulation.

iv) Carotid-sinus afferents and regulation of airway smooth muscle tone

In 1942, Seo and Nakayama (see 184) reported that bilateral resection of the carotid body (glomectomy) was a useful treatment for asthma. Since then, the presence of hyperresponsive chemoreceptor reflexes has been evoked occasionally to explain the etiology of asthmatic attacks. The technique was revived briefly in the 1960's, when bilateral and unilateral glomectomy or denervation of the carotid body was promoted as treatment for intractable asthma (184, 192, 223). Denervating the body invariably injured the innervation to the sinus, leading some to suggest that asthmatics have hyperreactive CCr and CBr reflexes (248). The technique went out of favor because the effects were only temporary in most individuals (248). Winter (272) "rerevived" the technique - after renaming the procedure for himself- and claims long-term success in patients with intractable asthma.

The role of CCr and CBr in the regulation of airway patency is not clear. In dogs and cats, Daly and Schweitzer (68) claimed that CSN stimulation can induce either bronchodilation or bronchoconstriction. The bronchoconstriction has been attributed to CBr stimulation, and the bronchodilation to CCr stimulation. In dogs, Nadel and Widdicombe

(182) found that CCr stimulation reflexly induced bronchoconstriction, whereas increasing ISP had no effect on bronchioles, but induced a small, yet significant dilation of the trachea. A recent study has confirmed this latter finding by showing that a decrease in ISP results in tracheal contraction (222). In guinea pigs, unilateral orthodromic electrical stimulation of the CSN induces a reflex bronchospasm via the sympathetic system (44).

Respiratory measurements in guinea pigs

Gas exchange requires a flow of air into and out of the lungs. In order to draw air into the lungs, the pressure within the alveoli (P_{alv}) must be lower than P_B. During inspiration, active contraction of the inspiratory muscles expands the thorax, thereby lowering intrathoracic pressure. The drop in intrathoracic pressure expands the alveoli, decreasing P_{alv} to a lower value than P_B, and air flows in. At end inspiration, the inspiratory muscles relax, withdrawing the force distending the lungs and the thorax, and the elastic tissues recoil. This elastic recoil returns the lungs and the thorax passively to the preinspiratory level (91, 170).

The size of the pressure gradient required to inflate the lungs is dependent on: 1) the frictional resistance during the movement of the tissues the lung and the thorax, 2) the resistance of the airways to airflow, 3) the elastic recoil of the lungs and the thorax, 4) the inertia of the volume of gas in the respiratory system (91). This is summarized by the equation of motion of the respiratory system (170):

$$\Delta P = \frac{V}{C} + R\dot{V} + I\dot{V} \qquad \text{Eqn. 1-1}$$

where ΔP is the airway pressure gradient; V is volume; $\dot{V}$ is airflow; $\ddot{V}$ is volume acceleration; I is viskance; R is resistance; C is compliance. The reciprocal of C, elastance (E) can be substituted into the equation. The pressure required to overcome inertia is commonly ignored because it accounts for less than 5% of the pressure gradient in a normal human at rest (14, 170).

Measurements of resistance are made by estimating the pressure exerted against resistance and the resulting rate of flow. This resistive pressure may be calculated by one of three ways. One method requires the measurement of the total pressure gradient, subtracting the pressure due to the elastic component. This subtraction is commonly performed by utilizing an x-y recorder, in which $\dot{V}$ appears on the Y axis and ΔP appears on the x axis. A single breath produces a closed loop. The points of intersection of the loop with the y axis correspond to points of zero flow, occurring between phases of respiration. The distance along the x axis of the loop is equal to elastic pressure. By superimposing a voltage corresponding to V on the y axis, the loop is closed and a curvilinear relationship between $\dot{V}$ and ΔP is obtained. Estimating the slope of this relationship yields the R value (14). A similar calculation can now be performed rather easily using a digital computer and least-squares fitting method (257). The latter method is described in more detail in the "Materials and Methods" section. Alternatively, ΔP may be measured at the two points of equal volume during a single breath. At this "isovolume" points, the elastic pressures are equal, thus any difference in ΔP reflects R. In the case of pulmonary resistance (R_L), transpulmonary pressure (P_{tp}) is used as the pressure gradient. Pulmonary resistance is the sum of the tissue resistance of the lung and the airflow resistance. Thoracic resistance (R_T) is the sum of R_L and tissue resistance of the chest wall, and is determined when transthoracic pressure is used as the pressure gradient. Resistance to airflow - airway resistance (R_{aw}) - is determined by measuring the pressure difference between the airway opening and the alveoli during airflow (14, 91).

Both static and dynamic compliance may be measured. Static compliance measures elastic forces only. It may be measured from the slope of a static P vs V curve. Dynamic compliance includes the elastic pressure and "elastic resistance" (14) and may be measured by subtracting the resistance pressure or by dividing V by P at points of zero airflow. Alternatively, if R has been derived, dynamic compliance may be calculated using a least-squares fit of the equation of motion of the lung (257). Pulmonary compliance (C_L) is determined when P_{tp} is measured; thoracic or respiratory system compliance is determined when transthoracic pressure is measured. The relationship between thoracic compliance (C_T), chest wall compliance (C_{cw}), and C_L is shown in the following equation:

$$\frac{1}{C_T} = \frac{1}{C_{cw}} + \frac{1}{C_L} \qquad \text{Eqn. 1-2}$$

Dynamic compliance is usually abbreviated C_{dyn} to distinguish it from static compliance. However, since only dynamic compliance was used in this thesis, C and its reciprocal E will stand for dynamic compliance and elastance, respectively.

Tests of lung mechanics in humans have been reviewed (14, 91). Similar techniques are used in the study of pulmonary mechanics of large and small animals with some modification. The tests of lung mechanics used in this thesis are described in the "Materials and Methods" chapter and will not be discussed here.

Thesis Proposal

Preamble

The list of drugs and chemicals capable of inducing asthma-like attacks is still growing. These bronchospastic agents act in different ways. Some appear to cause asthma by inducing anaphylactic reactions or by irritating the mucosa locally, whereas others can induce asthmatic reactions as idiosyncratic responses to the "normal" pharmacological actions of a drug (211).

Among the drugs which fall into the latter category are acetylsalicylic acid (ASA) and related non-steroidal anti-inflammatory drugs (NSAID) (129). About 10% of adults with asthma show intolerance to ASA and other NSAID, manifested as bronchospasm (243, 244). Tartrazine (TZ), a yellow azo-dye used as a food coloring, may also induce bronchospasm in ASA-intolerant asthmatics (211). However, this is now believed to be a rare phenomenon (243, 244). Precipitation of asthmatic attacks is thought to result from the inhibition of cyclooxygenase, which leads to an imbalance of eicosanoids (229, 244). Acetylsalicylic acid, and other NSAID which precipitate bronchospasm, have anti-cyclooxygenase activity. Non-steroidal anti-inflammatory drugs lacking anti-cylcooxygenase activity are not bronchospastic (243, 244). It is of interest that TZ has no anti-cyclooxygenase activity (244).

Using the guinea pig as a model, it has been shown that CSN afferents play a significant role in the reflex bronchoconstriction induced by NSAID and TZ (41, 202). NSAID and TZ (i.v.) induce a non-vagal, non-cholinergic reflex bronchoconstriction which is blocked by bilateral glossopharyngealectomy, sodium cromoglycate, and catecholamine depletors. Also, diazoxide and 5-HT have been shown to act via CSN afferents to induce a reflex bronchoconstriction which is similar to that induced by unilateral orthodromic electrical stimulation of the CSN in guinea pigs (43). The reflex bronchoconstriction induced by CSN stimulation is also mediated by non-vagal, presumably sympathetic, efferents. The bronchoconstriction may be pharmacologically differentiated into two components: one which is blocked by sodium cromoglycate and mepyramine, and another which is blocked by atropine (201).

General Hypothesis

That, in guinea pigs, the effects of CBr and CCr on respiration and lung mechanics are unknown and they, in conjunction with the sympathetic nervous system, could contribute to drug-induced bronchoconstriction in this species.

The general hypothesis stated above should be regarded as the theme for the series of studies described in this thesis. The experiments described here were designed to clarify the roles of CSN afferents and sympathetic efferents in the regulation of airway function in guinea pigs. Within this framework, the following questions were posed:

1. Do NSAID and TZ act directly on CSN nerve afferents to increase CSN activity?

2. If they do, is this action limited to a single receptor type,

CBr or CCr, or are both affected?

3. Does bilateral electrical stimulation of the CSN evoke the same reflex bronchoconstriction reported in response to unilateral electrical stimulation of this nerve?

4. What role, if any, do CCr and CBr afferents play in regulating bronchomotor tone, and is this role consistent with the actions of NSAID and TZ on these afferents?

5. Does an increase in systemic arterial blood pressure induced by pressor agents such as angiotensin II or phenylephrine alter airway tone? Is this response mediated or affected by CBr?

6. Do sympathomimetics increase or decrease airway tone in healthy guinea pigs? Is their action altered or exacerbated in "sick" animals sensitized to ovalbumin, and repeatedly exposed to ovalbumin?

Rationale for using guinea pigs

Guinea pigs were used for evaluating actions of agents on airway smooth muscle because:

1) The reflex bronchoconstriction induced by ASA, NSAID, and TZ is present in guinea pigs.
2) The sensitivity of guinea-pig airways to bronchoconstrictors and bronchodilators is similar to human airways.
3) Ovalbumin-sensitized guinea pigs are a good experimental model for asthma.

CHAPTER II

Materials and Methods

Animals

Hartley strain guinea pigs of either sex (400-700 g, body weight [b.w.]) were obtained from Charles River Canada, Ltd. (St-Constant, Quebec). The animals, transported in filter-top boxes, were housed on grids in cages suspended over trays containing rock salt, in laminar flow units (Bioclean, Hazelton Systems, Aberdeen, Md.) which supplied HEPA filtered air at positive pressure. These measures ensured that animals were not exposed to dust, allergens, or potential respiratory pathogens, or to chemical irritants such as ammonia. At necroscopy, animals' lungs were always free from respiratory infections. Light and electron microscopic examination confirmed this observation (W. C. Hulbert, personal communication). These precautions were taken to ensure that normal animals used in these experiments were free from respiratory diseases, and that they had not been exposed to pulmonary irritants while housed at the University of Alberta.

Anesthesia

Animals were anesthetized with urethane (1.25-1.50 g/kg, b.w., i.p.), pentobarbital (30-35 mg/kg, b.w., i.p.), or α-chloralose (80-120 mg/kg, b.w., i.p.) or, after induction with methoxyflurane, with α-chloralose (50-80 mg/kg, b.w., i.v.). In all animals, the trachea was cannulated (PE240) immediately below the larynx via a midline longitudinal incision along the ventral side of the neck from the level of the bulla to about 0.5 cm above the thoracic inlet.

Recording of spontaneous neural activity from intact carotid-sinus nerves

Neurophysiological studies were performed on spontaneously breathing guinea pigs anesthetized with urethane. Figure 2-1 shows the anatomy of the carotid bifurcation in the guinea pig. The common carotid artery (CCA) was isolated on either side as it divides into the occipital artery (OA) and the external carotid artery (ECA); the guinea pig does not have an internal carotid artery (206). The carotid sinus was isolated at the base of the OA. The carotid-sinus nerve (CSN) was isolated as it exited the carotid sinus/carotid body region apposed to the branch of the anterior pharyngeal artery (APA). The CSN was dissected free, cut close to its junction with the glossopharyngeal nerve (IX), and desheathed. The nerve is extremely fine and less than 2 cm in length. Mineral oil was applied over the entire exposed area to reduce current spread and to keep the tissue moist. Carotid-sinus-nerve activity was recorded with bipolar platinum electrodes connected via a high impedance probe to a variable gain amplifier and band-pass filter (0.08-1.00 kHz). It was quantified by rectifying (full-wave) and integrating signals above the noise level at 1-s intervals, using a timer-reset integrator. Output from the amplifier was fed to a loudspeaker, and was also displayed continuously with the inverted integrated signal on a dual-beam oscilloscope (Tektronix 5113). Integrated CSN activity, and the mean arterial blood pressure (MABP) were digitized every 1 s, averaged, and displayed every 5 s (Buxco DL12 Data Logger, Texas Instruments Silent 700 printer).

Isolated perfused carotid sinus for neurophysiological studies of the effects of NSAID and TZ on CSN-afferents

i) Surgical Preparation

The carotid sinus on either side was exposed, its CSN was dissected free and prepared for recording of spontaneous discharge as described above. If the nerve showed characteristic pulsatile firing in synchrony with systole, it was removed from the electrodes and carefully reflected to one side to prevent damage during isolation of the sinus. The carotid sinus was isolated vascularly in situ by ligating (6-0 silk) the OA, the APA, the thyroid, the lingual, and any small tributary arteries distal to the sinus. A cannula (PE60) was placed in the ECA to act as an outlet for the perfusate and to monitor intrasinus pressure (ISP) (Gould P50 pressure transducer, Buxco Cardiovascular Analyzer). A second cannula was placed towards the sinus with its tip in the CCA and was connected to a Marriott bottle containing Krebs solution at 37°C equilibrated with 95% O_2/5% CO_2.

The sinus was perfused at a flow rate of 2 ml/min and an ISP of 15 mm Hg. Pressure in the Marriott bottle, and thus in the sinus, was controlled by the gas regulator attached to the pressurized source of 95% O_2/5% CO_2, and by a screw clamp which acted as a variable load on the ECA outflow. The sympathetic innervation to the sinus was sectioned close to the superior cervical ganglion and the contralateral sinus was denervated. A diagram of the perfusion system is shown in Figure 2-2.

It should be pointed out that the sinus was not isolated from the carotid body, because the body, which is located at the origin of the

APA, is contiguous with the sinus (60). On two occasions, 0.05 ml of 0.15 N acetic acid was infused into the isolated sinus to inactivate CCr (96).

ii) Protocol to generate ISP-CSN activity curves

After completing the surgical procedure, the CSN was placed on the recording electrodes and CSN-activity allowed to stabilize over a 5 to 10 min period. Then the ECA outflow and the gas regulator were closed, effectively making the isolated sinus a 'blind sac', and the pressure in the Marriott bottle, and thus ISP, reduced to 0 mm Hg with respect to atmosphere. Using a large (30 ml) air-filled syringe as a pressure source, ISP was then slowly raised in a ramp until CSN activity increased. The ISP at this point was taken to be baroreceptor threshold. Once the threshold value was determined, ISP was raised to nearest multiple of 10 mm Hg and then increased stepwise in increments of 10 mm Hg at 30-50 s intervals until CSN activity no longer increased with the rise in ISP. Stepwise increases in ISP induce an initial increase in CSN activity which rapidly adapt to a new level (145). The latter level of activity is called steady-state discharge. In this series of experiments, CSN activity reached steady-state within 10-30 s of each stepped increase in pressure. The steady-state values were used for data analysis. Each ISP-CSN activity stimulus-response curve was generated in about 5 min. After the stimulus-response curve had been generated, the ECA outflow was opened and the sinus was perfused for a minimum of 15 min.

In each animal studied, at least two consecutive ISP-CSN activity, stimulus-response curves were generated within 30 min of isolating the sinus. The two curves were compared by analysis of variance for comparison of two regression lines (236). The effects of drugs on the ISP-CSN activity relationship were examined only if two consecutive stimulus-response curves generated within 30 min of isolation differed by < 5%. Drug solutions were perfused for 5 min at 2 ml/min (ISP = 15 mm Hg). The ECA was then closed and the ISP-CSN activity relationship in the presence of the drug was examined. After exposure to a drug solution, the sinus was perfused for at least 15 min with Krebs solution. An isolated carotid sinus usually gave reproducible responses for 2.5 h. All experiments were completed within 2 hrs. The protocol is summarized in Figure 2-3.

iii) Analysis of ISP-CSN activity curves

Least-squares linear regression was performed on the linear portion of the curve relating CSN activity to ISP (ISP range from 30-70 mm Hg) and the slope of the regression line was used as an index of baroreceptor sensitivity or gain (111). Analysis of variance was used to compare threshold and saturation pressures and slopes following administration of drugs (236). Differences were considered significant at the 5% level.

Selective physiological stimulation of carotid baroreceptors

Carotid baroreceptors were stimulated selectively in two different preparations. In the first, the left and the right carotid sinuses were isolated and perfused in tandem with Krebs solution as described above. Both CSN were left intact, but the aortic depressor nerves (ADN) were sectioned on both sides.

Previous experience with the isolated sinus had shown that the system was susceptible to small leaks and it was impossible to physically separate the carotid body from the vascular supply of the sinus. In order to study the CBr reflexes in isolation, it was imperative to remove or control CCr input. In rabbits, Franz (92) showed that a suspension of talc (300 mg/ml) in Krebs solution injected into the carotid sinus/carotid body produced thrombosis in the small vessels supplying the body and eliminated chemoreceptor activity probably by inducing permanent anoxia. The talc also helped to prevent leaks.

After isolating both sinuses, a talc suspension (300 mg/ml) was injected into both CCAs until it could be seen at the ECA outflow. Then after closing the outflow, ISP was raised to 50 mm Hg for 3 min, and the ECA reopened and the sinuses flushed with Krebs solution for 20 min at a flow rate of 2 ml/min (ISP = 15 mm Hg). The effectiveness of the procedure was determined by comparing responses to injection of (50 μg/kg NaCN) in 0.1 ml Krebs into the isolated sinuses before and after infusion of the talc suspension.

Following a 10 min washout, the ECA outflow and the gas regulator valves were closed, ISP was raised from 50 mm Hg to 100 mm Hg, maintained for 30 s and then reduced to baseline of 50 mm Hg. Steady-state values of R, E, Vt, f, HR, and MABP were recorded at each ISP. This preparation gave stable and reproducible results for 1.5-2.0 h.

The second preparation used to stimulate CBr selectively is shown schematically in Figure 2-3. The major difference between this system and that described above was the use of the animal's own blood to perfuse the sinuses. The sinuses and CSN were isolated and exposed as before. The CCA were tied and cut approximately 2 cm from each sinus and cannulas (PE 50) placed towards the sinuses on both sides. The sinuses were then attached to the outflow of a peristaltic pump (Masterflex Portable, Cole-Parmer). Another cannula (PE 60 or 90) was placed in the left CCA towards the heart. The right CCA was also cannulated (PE 60) to monitor blood pressure (Gould P50 pressure transducer; Grass Physiograph). This system is shown schematically in Figure 2-4. The blood was pumped into the sinuses at a flow rate of 2-4 ml/min and drained via the ECA into the right jugular vein. A screw clamp placed between the ECA and the external jugular vein provided variable load. Intrasinus pressure was monitored via a pressure transducer placed before the screw clamp. The total dead volume of the extracorporeal perfusion system was 1.2 ml. Guinea pigs were heparinized (150 U/kg) before starting perfusion and all cannulas and tubes were loaded with heparinized 0.9% NaCl solution (50 U/ml). End-tidal $[CO_2]$ was monitored continuously (Spectralab-M mass spectrometer, VG Gas Analysis). Animals were allowed to breathe freely O_2 supplied by free flow via an oversized tube place loosely over the unobstructed port of the pneumotachograph. Carotid baroreceptors were stimulated by increasing ISP in a single step of 50 mm Hg from a baseline of 50 mm Hg.

Selective stimulation of carotid chemoreceptors

Cannulas were placed into the ECA on both sides as described above. They were connected to each other and NaCN (50-200 µg/kg) in 0.1 ml 0.9% saline was injected retrogradely via a 0.5 ml syringe.

Bilateral electrical stimulation of the CSN

The CSN on both sides were identified, dissected free from the surrounding tissue, and cut proximal to the sinus. The nerves were placed on bipolar electrodes and the entire area was flooded with mineral oil. The ADN were sectioned on both sides. CSN were stimulated at 2-4 Volts, 0.05-0.40 ms pulse width at 5 Hz for 10 s.

Determination of pulmonary airflow resistance and dynamic pulmonary compliance in spontaneously respiring animals

Pulmonary airflow resistance (R_L) and dynamic pulmonary compliance (C_L) or elastance (E_L) were determined: (1) manually using the method of Amdur and Mead (10) and, (2) with a computer using a least-squares-fitting technique described by Uhl and Lewis (257). Both methods use the following equation derived from a mechanical model of the lung (170):

$$P_{tp} = \frac{V}{C_L} + R_L\dot{V} \qquad \text{Eqn. 2-1.}$$

The determination of $\dot{V}$, P_{tp}, and V will be described after the methods used to calculate R_L and C_L have been outlined.

i) Manual determination of R_L and C_L in spontaneously respiring animals.

The manual method for calculating R_L and C_L was first proposed by Amdur and Mead (10), who showed that information on the mechanical properties of the lungs may be obtained by relating V and $\dot{V}$ to P_{tp} at specific points in the respiratory cycle. This isolated points method is summarized in Figure 2-5. At points of zero airflow, such as the beginning and end of inspiration, the changes in P_{tp} relate solely to elastic forces. Thus, C_L may be calculated by dividing the change in V from the beginning to the end of inspiration, i.e. V_t, by the change in P_{tp} over this period:

$$C_L = \frac{V_t}{P_{tp}} \qquad \text{Eqn. 2-2.}$$

Pulmonary elastance was determined by taking the reciprocal of C_L. Similarly, at points of equal volume during a breath, the elastic forces must be approximately equal, and the change in pressure between two such points must relate to airflow resistance:

$$R_L = \frac{P_{tp}}{\dot{V}} \qquad \text{Eqn. 2-3.}$$

Mid-tidal volume was used for the isovolume point.

ii) Computer-assisted least-squares fitting technique for estimating R_L and C_L.

Unlike the Amdur-Mead method, the least-squares-fitting method estimates R_L and C_L using P_{tp}, $\dot{V}$, and V points along the entire breath and is thus less susceptible to error (257). In essence the least-squares-fitting method acts to minimize the ΣS of the squared error between the observed pressure wave (P_{obs}) and a best fit curve ($P_{best\ fit}$):

$$S = (P_{best\ fit} - P_{obs})^2 \qquad \text{Eqn. 2-4.}$$

Equation 2-1 states that

$$P_{obs} = \frac{V}{C} + R\dot{V} \qquad \text{Eqn. 2-5.}$$

Substituting 2-5 into 2-4 yields

$$\Sigma S = \left[P_{\text{best fit}} - \frac{V}{C_L} + R_L\dot{V} \right]^2 \qquad \text{Eqn. 2-6.}$$

Expanding 2-6, and taking the partial derivatives $\delta S/\delta(^1/_{CL})$ and $\delta S/\delta R_L$ to minimize the error, produces the following solutions for R_L and C_L:

$$R_L = \frac{\Sigma V^2\, \Sigma P\dot{V} - \Sigma PV\, \Sigma V\dot{V}}{\Sigma V^2\, \Sigma\dot{V} - \Sigma(V\dot{V})^2} \qquad \text{Eqn. 2-7}$$

$$C_L = \frac{\Sigma V^2}{\Sigma PV - R\, \Sigma V\dot{V}} \qquad \text{Eqn. 2-8.}$$

R_L and C_L were calculated using the above equations by a MINC23 digital computer (Digital Equipment). The reciprocal of C_L was calculated by the computer to yield E_L. The computer samples and converts analog P_{tp} and $\dot{V}$ signals into a digital array at a rate of 50 data points/s and stores them in a memory with a maximum capacity of 29,999 points.

After sampling and storing the data, the memory is accessed and the data points are separated into subgroups for every breath. The procedure followed by the program to identify a breath is shown in Figure 2-6. First, the computer scans forward in time in the V array to find a positive data value greater than a predetermined "level". Recognizing this point as a potential breath, the computer logic continues to search forward for a point of zero flow, an indication of the probable end of inspiration. Then the computer scans forward to find a negative data point less than a predetermined "level", indicating a probable expiration. Once such a point is located the computer searches forward in time for a zero flow point, the end of expiration. Returning to the inspiration and expiration "level" points, the computer searches backwards for zero flow points which it assumes to be the start of inspiration and expiration.

The number of points collected per breath varied with breathing rate. For each breath the computer calculated and consecutively stored the five summations: ΣV^2, $\Sigma P\dot{V}$, $\Sigma V\dot{V}$, $\Sigma\dot{V}^2$, and ΣPV, from the start of inspiration to the end of expiration. A smoothing function was not employed, as it was felt that such a function was unnecessary given the rate of digitization and would only detract from sensitivity. The computer program integrated $\dot{V}$ from the start to the end of inspiration to determine V_t. Respiratory frequency (f) was determined manually by counting the number of breaths per unit time.

Measurement of lung volumes in spontaneously breathing animals

A water-jacketed, constant-volume plethysmograph was used to determine lung volumes for manual calculation of R_L and C_L, and airway resistance R_{aw} and functional residual capacity (Fig. 2-7). The plethysmograph had an internal volume of 2.040 L (7.0 cm wide, 8.0 cm high, 36 cm long inside) and was constructed from Plexiglass. It connected to a water-jacketed bottle with an internal volume of 1.076 L. Temperature was maintained within the system by pumping water warmed to 37°C through the external jackets (Haake E51, temperature-controlled water pump). Volume was measured as the pressure change in the (airtight) plethysmograph (P_{box}) and detected with a calibrated pressure transducer (Grass PT5 transducer, 7P122B low-level DC Amplifier, Model 7D Physiograph). Calibrations were performed with the box empty. Air

(1-10 ml) from a calibrated syringe was introduced into the box, held for 3 s, and P_{box} signal displayed on the physiograph and on the x-axis of a storage oscilloscope (Tektronix 5113). A dynamic test was also performed. Air (2-6 ml) was introduced and withdrawn from the plethysmograph at a frequency of 0.33-1 Hz by connecting both the inlet and the outlet of a respirator (Small Rodent Respirator, Model 681, Harvard Apparatus) to the plethysmograph. The effect of volume displacement of the animal's body was corrected with a specific gravity factor of 1.08 g/ml, measured by water displacement.

Initially, the bottle was not connected to the plethysmograph, but the P_{box} signal fluctuated and never quite stabilized. The bottle was connected to the plethysmograph by a low-resistance tube to act as a reservoir. With the bottle in place, the P_{box} signal stabilized within 15-20 min of placing the animal in the box or opening the box to atmosphere. Others have used larger reservoirs filled with copper wire to establish isothermal conditions (147). In my experiments, the addition of the bottle and maintaining temperature at 37°C was sufficient to maintain isothermal conditions.

Measurement of airflow rates in spontaneously breathing animals

Airflow was measured via a screen pneumotachograph built into the plethysmograph. The pneumotachograph, shown in Figure 2-8, was designed by R.L. Jones and consisted of a double layer of 400 mesh stainless steel screen. It was connected to a differential pressure transducer (MP45 ± 2 cm H_2O, Validyne Engineering, Northridge, CA.; Buxco Validyne Flow Preamplifier). Air at various flow rates (5-20 ml/s determined by a rotameter (Matheson 603) was passed through the pneumotachograph to calibrate it.

Animals breathed through the pneumotachograph via a tracheal tube. A 3-way valve located between the double-thick screen and the air inlets was used to shut the airway opening and/or to determine whether the animals breathed air from the atmosphere or air from within the box. The combined dead space of the pneumotachograph and the breathing circuit was 1.15 ml.

Measurement of pleural and esophageal pressure in spontaneously breathing animals

Pleural pressure (P_{pl}) was used instead of transpulmonary pressure to determine R_L and E_L. Esophageal pressure (P_{es}) was used to estimate P_{pl} in all spontaneously breathing animals. Esophageal pressure was measured via a water-filled cannula (PE60) with four side openings within 1 cm of the end hole connected to a low-volume pressure transducer (Gould P50). The cannula was inserted into the stomach (as determined by positive - with respect to atmosphere - pressure fluctuations during spontaneous inspiration), and cleared by injecting 1-2 ml of water, and then it was slowly withdrawn until maximal negative pressure swings were observed in synchrony with respiration. Pleural pressure (Ppl) was measured via an air-filled trochar (16 gauge feeding tube, W.S. Popper) with three side holes within 1.0 cm of the end hole and compared with the P_{es} signal in 5 animals. The air-filled cannula was inserted into the pleural space through a mid-thoracic incision. The cannula was sealed to the chest wall with a purse-string suture.

Pneumothorax was minimized by withdrawing air from the pleural space with a syringe until tension was felt on the plunger, whereupon 0.3-0.5 ml of air was reinjected to separate the visceral and parietal pleura at the cannula tip. The cannula was connected to one arm of a differential pressure transducer (Validyne MP45 ± 50 cm H_2O), positioned until the negative pressure swings during inspiration were maximal, and then locked into place with epoxy (DEVCON 5 minute).

Measurement of functional residual capacity

Functional residual capacity (FRC) was measured by the method of Dubois _et al._ (82). Briefly, the animal was placed in the plethysmograph, and the tracheal cannula connected to the pneumotachograph and the valve assembly. The valve was then turned to occlude the airway opening, and P_{ao} and P_{box} were measured and displayed on x and y axis of the oscilloscope while the animal attempted to breathe against the closed opening. The angle (α) of P_{ao} - ΔV relationship was measured with a protractor and substituted into the following equation:

$$FRC = (P_B - 47\ \mathrm{mm\ Hg})\cot\alpha \left[\frac{3116 - (\mathrm{b.w.}/1.08)}{3116}\right] 0.0435\ \mathrm{ml\ mm\ Hg}^{-1} \qquad \text{Eqn. 2-9}$$

where 3116 ml is the volume of the plethysmograph and the reservoir, 1.08 g/ml is the specific gravity of a guinea pig, and 0.0435 is a calibration factor of the relationship of V/P_{ao}. The dead space of the breathing circuit (1.15 ml) was subtracted from the calculated volume to yield actual FRC.

Measurement of airway resistance

Airway resistance (R_{aw}) is the ratio of P_{alv} to $\dot{V}$ at a particular point in the respiratory cycle. It was measured using the method of Dubois et al. (83). A plethysmograph is required for this measurement to determine P_{alv}. The relationship between P_{alv} and P_{Box} was obtained by having the animal breathe against a closed airway. This manoeuvre is identical to that followed to determine FRC. Next, the valve on the pneumotachograph was set so the animal breathed from within the plethysmograph. During spontaneous breathing, a minimum of three P_{Box} versus $\dot{V}$ tracings were displayed on the storage oscilloscope. It should be noted that measuring R_{aw} requires that the animals breathe gas at or near body temperature and 100% relative humidity. These conditions were established by flushing air saturated with water vapor at 37°C through the plethysmograph and reservoir for about 5 min.

Airway resistance was computed by measuring the angle of the P_{box} - P_{alv} relationship (equivalent to α above) and of the $\dot{V}$ - P_{Box} relationship (β angle). As summarized in equation 2-10, R_{aw} is equal to the product of the tangent of the α angle and the cotangent of the β angle multiplied by calibration factors for the relationships of P_{alv}/P_{box} and $P_{box}/\dot{V}$, which is simplified to 1.67 cm $H_2O \cdot s \cdot ml^{-1}$.

$$R_{aw} = (\tan\alpha)\ (\cot\beta)\ 1.67\ \text{cm}\ H_2O.s.ml^{-1} \qquad \text{Eqn. 2-10.}$$

The resistance of the breathing circuit and the cannula (0.008 cm $H_2O \cdot s \cdot ml^{-1}$) was subtracted from this value to yield the actual R_{aw}. Specific airway conductance (sG_{aw}) was calculated as follows:

$$sG_{aw} = \frac{(1/R_{aw})}{FRC} \qquad \text{Eqn. 2-11.}$$

Determination of dynamic thoracic airflow resistance and dynamic thoracic elastance in artificially-ventilated animals

The methods followed to calculate thoracic resistance and dynamic thoracic elastance in artificially-ventilated guinea pigs have been described by Goel (98). Briefly, guinea pigs were tracheotomized (PE240) and the tracheal cannula was connected via a pneumotachograph (Fleisch 0000) to a Harvard small animal respirator modified for solenoid-operated valves. Animals were paralyzed with decamethonium (10 mg/kg) and ventilated at a frequency of 0.33 Hz and V_t adjusted to maintain an end-tidal CO_2 of 4.5 % (Spectralab-M mass spectrometer, VG Gas Analysis). The pneumotachograph was connected to a differential transducer (Validyne MP45 ± 2 cm H_2O) to measure $\dot{V}$. A second differential transducer (Validyne MP45 ± 50 cm H_2O) was connected to a side arm of the tracheal cannula to measure intratracheal pressure. Intratracheal pressure and $\dot{V}$ signals were recorded only during inspiration. The outlet valve was placed proximal to the animal and opened during expiration. Therefore, neither $\dot{V}$ nor P were monitored during expiration. Resistance and elastance were calculated during inspiration by the MINC computer using the equations of Uhl and Lewis (257). Tracheal pressure was greater than atmosphere and gave an estimate of transthoracic pressure, thus the system calculates dynamic thoracic elastance (E_T) and not E_L. Goel (98) states that the resistance calculated is pulmonary; however, since tracheal pressure estimates transthoracic pressure, the resistance value will include some measure of the viskance of the chest wall. Thus, the resistance values calculated in artificially-ventilated animals will be termed thoracic resistance (R_T).

Measurement of arterial blood pressure

Mean arterial blood pressure (MABP) was monitored from either a femoral, an axillary, or a carotid artery. Carotid and femoral arteries were cannulated using a PE90 catheter with a tapered tip, axillary arteries were cannulated using a more flexible PE50 catheter. In most experiments MABP was recorded via a Gould P50 pressure transducer (HewlettPackard 7805 polygraph or a Grass 7D polygraph). In neurophysiological studies, MABP was recorded using a P50 transducer and Buxco Cardiovascular Analyzer. The signal was digitized over 1 s, averaged, and displayed every 5 s (Buxco Data Logger DL12; Texas Instruments Silent 700 printer).

Measurement of heart rate

Heart rate (HR) was determined from the ECG (QRS complex), using the equivalent of human lead II (Hewlett-Packard rate meter and Bioelectric Amplifier).

Chemical sympathectomy

Twenty-four hours before experiments, animals were given reserpine (2.5 mg/kg, i.p.) or 6-hydroxydopamine (35.0 mg/kg, i.p.). Sympathectomy was assumed to be complete if tyramine (0.25 mg/kg, i.v.) failed to increase MABP.

Ovalbumin sensitization

The protocol used to sensitize guinea pigs to ovalbumin was similar to that described by Barnes et al. (25) and Mita et al. (172). Guinea pigs were allocated randomly to one of two groups. One group of eight animals, received an injection of ovalbumin (5 mg/kg, i.p.) in 0.5 ml 0.9% saline on day 1, and a second injection of ovalbumin (10 mg/kg, i.p.) in 1.0 ml 0.9% saline on day 3. The second group of four animals received 0.5 ml of 0.9% saline on day 1 and 1.0 ml of 0.9% saline on day 3. Three weeks after the initial injections both groups commenced aerosol treatments. The control group was exposed daily for 14 days to 0.9% saline aerosol for 8 min. The animals in the test group were exposed daily over the same time period to an aerosol of a 1% solution of ovalbumin in 0.9% saline for 8 minutes, or until they showed dyspnea or coughing.

Nerve sections

The vagi were isolated from the surrounding tissue and sectioned at mid-cervical level. The ADN were sectioned at their junction with the superior laryngeal nerve (Figure 2-1)

Drugs and solutions

i) General

A cannula (PE50) was placed in an external jugular vein for intravenous (i.v.) injection of drugs. The dead volume of the cannula was 0.10-0.15 ml. All drugs were dissolved in, or diluted with, 0.9% saline. Whenever possible the drugs were injected in volumes of less than 0.2 ml and the cannula was flushed with 0.2 ml of saline after each drug injection. Unless otherwise noted, all doses were adjusted for b.w.

ii) α-Chloralose

α-Chloralose (10 mg/ml, Sigma) was dissolved in a 10% solution of polyethylene glycol 200 in saline at 45°C, or in saline warmed to 65-70°C.

iii) Prazosin Hydrochloride

Prazosin hydrochloride (0.5 mg/ml) (gift from Pfizer Pharmaceuticals) was dissolved in ultrapure 18 megohm water warmed to 40°C.

iv) Other Drugs

All other drugs were soluble in normal saline. Drugs used were: acetylsalicylic acid (Mallinckrodt Inc.), human angiotensin II and III (Peninsula Laboratories), atropine sulfate (Fluka AG), decamethonium bromide U.S.P., hexamethonium bromide (K & K Fine Pharmaceuticals), 6-hydroxydopamine (Sigma), indomethacin sodium trihydrate (a gift from Merck, Sharp, and Dohme Canada), α-methylnoradrenaline (nordefrin, a gift from Winthrop Laboratories), mepyramine maleate (a gift from Rhône Poulenc Pharma. Inc.), NaCN technical grade (General Intermediaries, Canada), L-noradrenaline bitartrate (Fluka AG), ovalbumin grade VI

(Sigma), pentobarbital (Euthanyl^R, M.T.C. Pharmaceuticals), phentolamine hydrochloride (Rogitine, Geigy Pharmaceuticals), phenylephrine hydrochloride U.S.P., reserpine injection (Serpasil^R, Geigy Pharmaceuticals), sodium cromoglycate (a gift from Fisons Corporation, Ltd.), tartrazine U.S.P., tyramine hydrochloride (Fluka AG), urethane (Sigma), sar^1-val^5-ala^8-angiotensin II (Peninsula Laboratories), and yohimbine hydrochloride (Sigma).

v) Krebs Solution

Krebs physiological solution was made fresh daily and contained the following: 118 mM NaCl, 4.7 mM KCl, 1.1 mM KH_2PO_4, 1.1 mM $MgSO_4$, 2.5 mM $CaCl_2$, 24 mM $NaHCO_3$, and 10 mM dextrose. The solution was equilibrated at 37°C with 95% O_2/5% CO_2 for 2 h before use.

Experimental design and analysis of data

i) Experimental design

Experiments involving the generation of dose-response curves were based on a randomized-block design with each animal acting as its own control. Dose-response curves for agonists (i.v.) were generated by administering at least three doses in random order. The effects of blockers or denervations on agonist-induced responses were examined by generating dose-response curves for an agonist before and after treatment. A minimum of 4 animals was used for a given treatment. The effects of pretreatments, such as chemical sympathectomy or ovalbumin-sensitization, were evaluated by comparing dose-response curves generated in the treated group with those generated before the addition of blockers or the sectioning of nerves, or with those generated in sham-

or saline-treated groups, respectively.

ii) Analysis of data

Coefficient of correlation (r) was used to examine dose-dependency and the relationships among parameters. Slopes of log dose-response curves were determined by least-squares analysis. Analysis of variance (ANOVA) was used to verify dose-dependency and to evaluate the effects of blockers and nerve sections. Each dose or blocker or nerve section was designated as a treatment and ANOVA was used to determine the significance of the treatments. Effects of various anesthetics on baseline data were compared by Student's t-test (236). BMDP Statistical Software package was used to analyze the data (75). Differences were assumed significant at the 5% level. Data are presented as mean ± s.e.m.

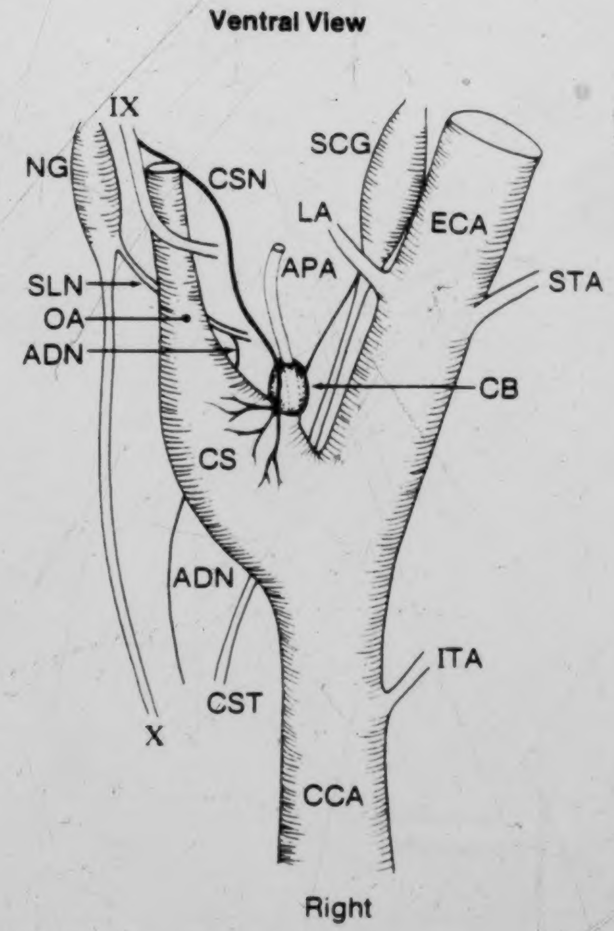
Ventral View
IX
NG
CSN
SCG
LA
ECA
APA
STA
SLN
OA
ADN
CB
CS
ADN
ITA
CST
X
CCA
Right

Figure 2-1. Diagram of the right carotid bifurcation region (ventral view) showing the location of the carotid body (CB), carotid sinus (CS) and the nodose (NG) and superior cervical ganglion (SCG). The diagram also shows the paths of the carotid-sinus (CSN), aortic depressor (ADN), glossopharyngeal (IX), and vagus nerves (X) and their relationship to the CCA and its tributaries in the guinea pig. Note the absence of an internal carotid artery.

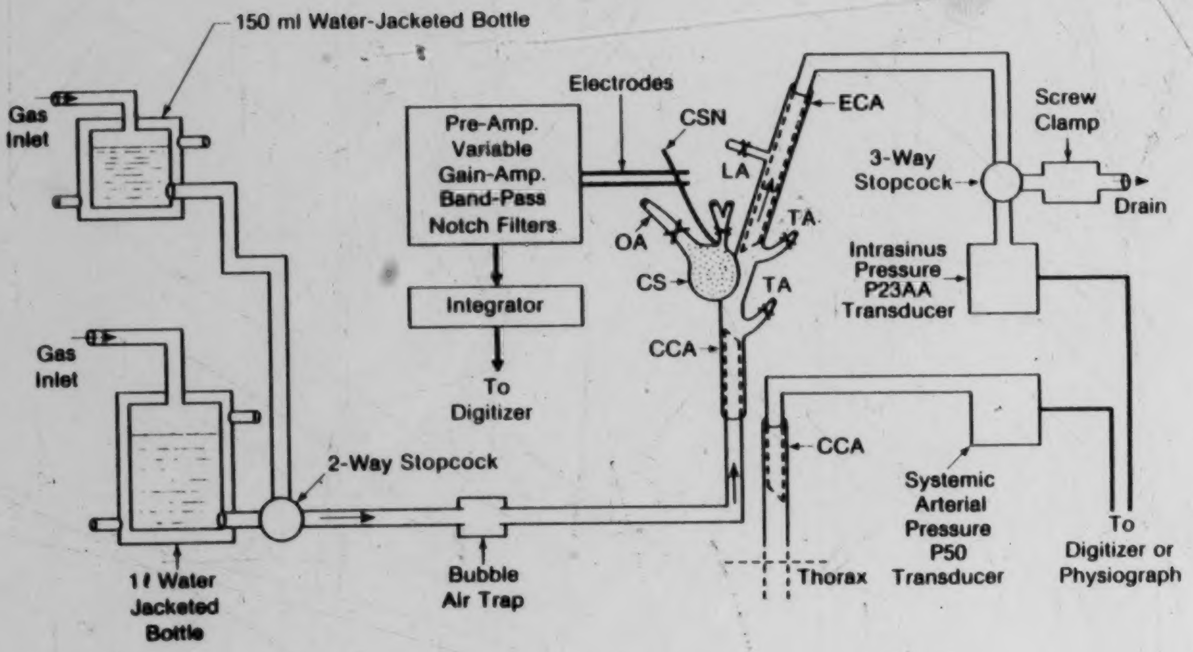
150 ml Water-Jacketed Bottle
Gas Inlet
Gas Inlet
1 ℓ Water Jacketed Bottle
2-Way Stopcock
Bubble Air Trap
Pre-Amp. Variable Gain-Amp. Band-Pass Notch Filters
Integrator
To Digitizer
Electrodes
CSN
OA
CS
CCA
LA
TA
TA
ECA
CCA
Thorax
3-Way Stopcock
Screw Clamp
Drain
Intrasinus Pressure P23AA Transducer
Systemic Arterial Pressure P50 Transducer
To Digitizer or Physiograph

Figure 2-2: Schematic of the system used to perfuse isolated sinus with Krebs Solution.

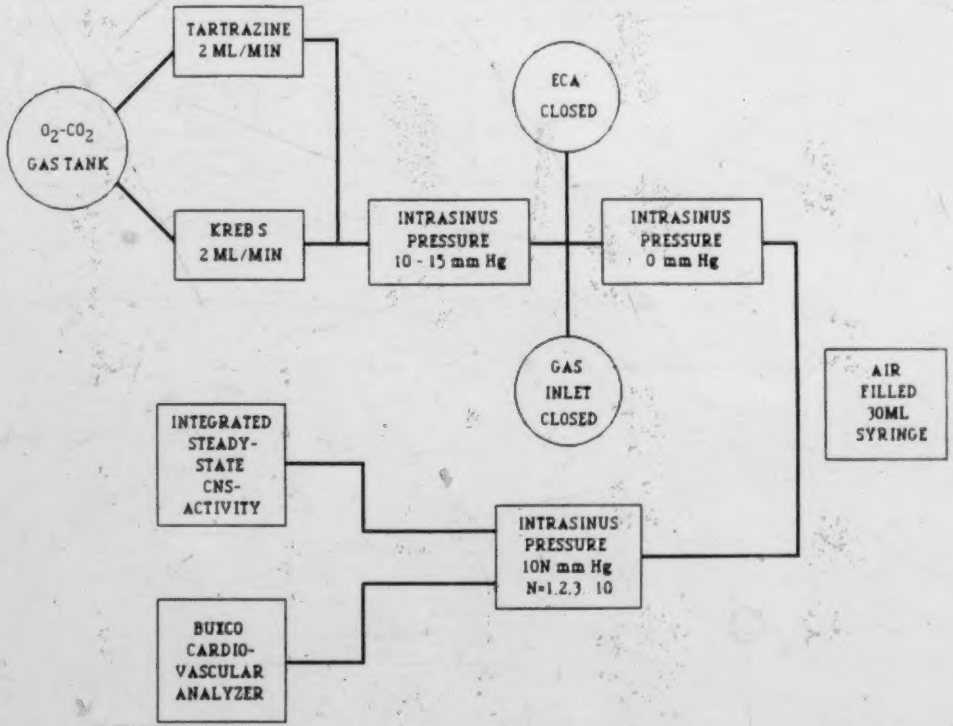
TARTRAZINE
2 ML/MIN
O_2-CO_2
GAS TANK
KREBS
2 ML/MIN
INTRASINUS
PRESSURE
10 - 15 mm Hg
ECA
CLOSED
INTRASINUS
PRESSURE
0 mm Hg
GAS
INLET
CLOSED
AIR
FILLED
30ML
SYRINGE
INTEGRATED
STEADY-
STATE
CNS-
ACTIVITY
INTRASINUS
PRESSURE
10N mm Hg
N=1,2,3 10
BUXCO
CARDIO-
VASCULAR
ANALYZER

Figure 2-3: Protocol followed to generate curves relating ISP to CSN activity.

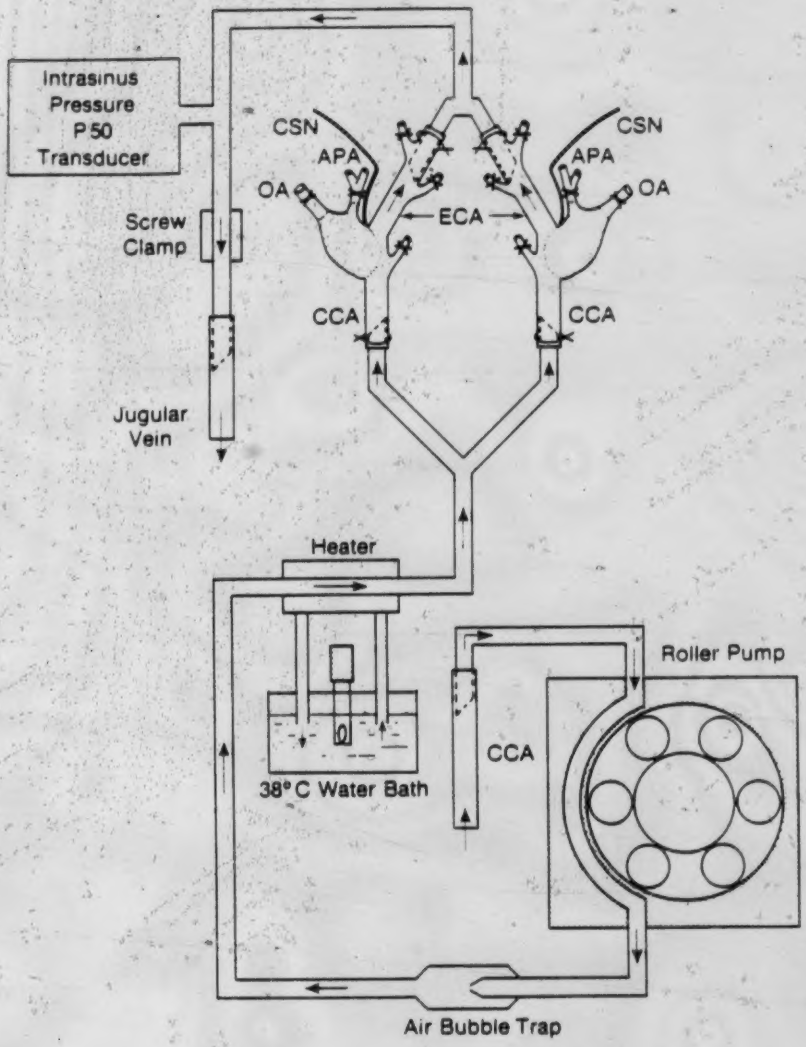
Intrasinus
Pressure
P50
Transducer
Screw
Clamp
Jugular
Vein
CSN
APA
OA
ECA
CCA
CSN
APA
OA
CCA
Heater
38° C Water Bath
CCA
Roller Pump
Air Bubble Trap

Figure 2-4: Schematic of the system used to perfuse carotid sinuses in tandem with the animal's own blood.

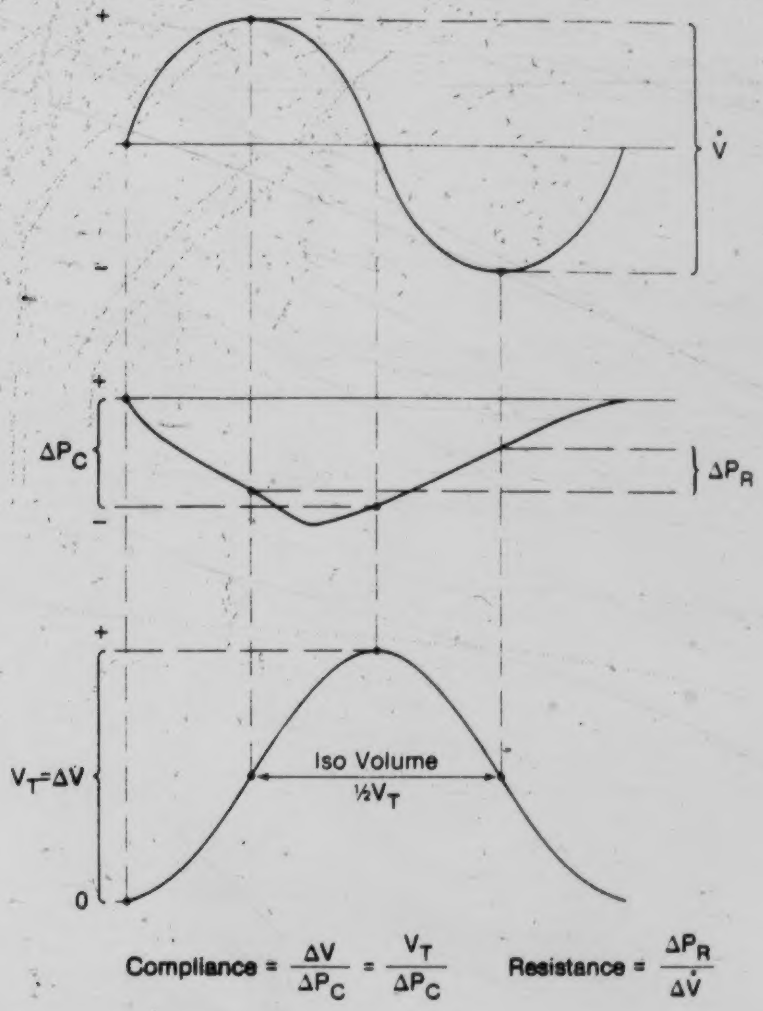
+
−
$\dot{V}$
+
ΔP_C
−
ΔP_R
+
$V_T = \Delta V$
Iso Volume
$\frac{1}{2}V_T$
0
Compliance = $\frac{\Delta V}{\Delta P_C} = \frac{V_T}{\Delta P_C}$
Resistance = $\frac{\Delta P_R}{\Delta \dot{V}}$

Figure 2-5. Isolated points method first described by Amdur and Mead (10). Compliance is calculated from the beginning to the end of inspiration. Resistance is calculated at mid-tidal volume ,but may be calculated at any two isovolume points.

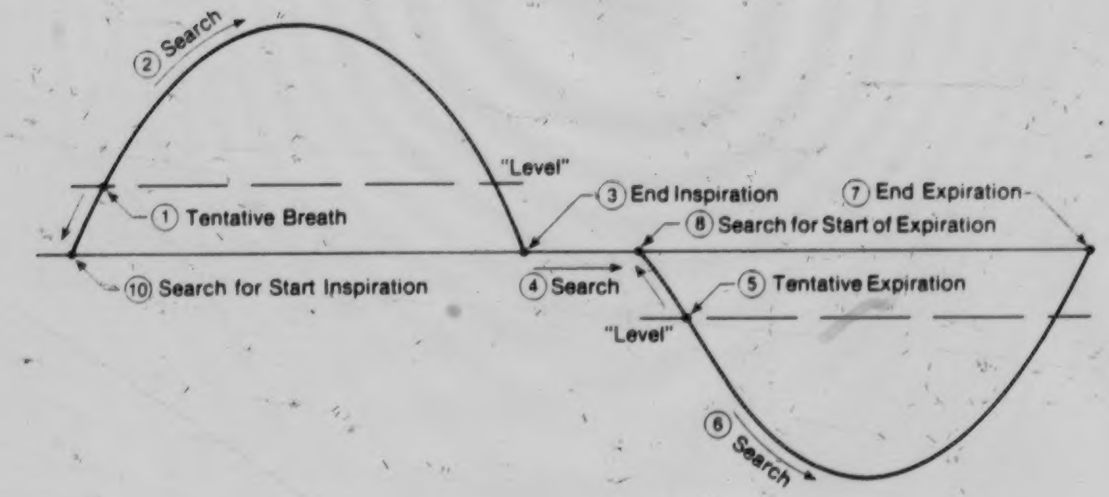
② Search
"Level"
① Tentative Breath
③ End Inspiration
⑦ End Expiration
⑧ Search for Start of Expiration
⑩ Search for Start Inspiration
④ Search
⑤ Tentative Expiration
"Level"
⑥ Search

Figure 2-6. Schematic of the protocol used by the the digital computer to identify one breath (adapted from 257).

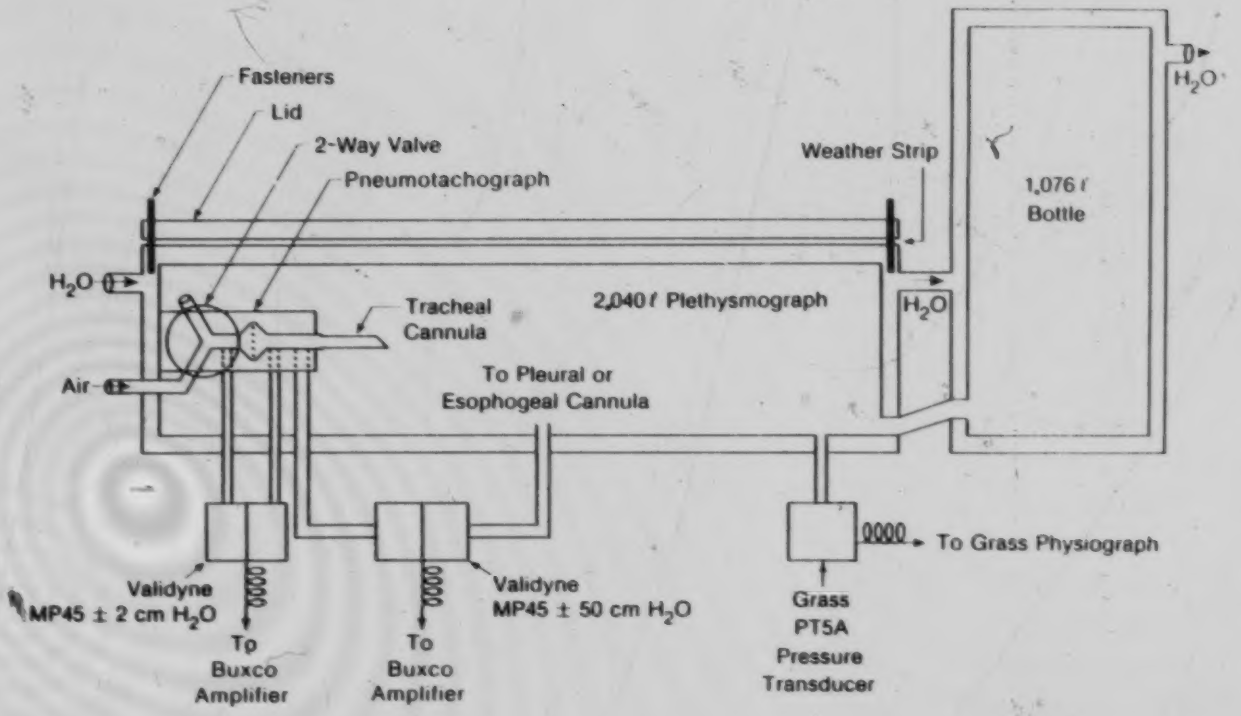
Fasteners
Lid
2-Way Valve
Pneumotachograph
Weather Strip
1,076 ℓ
Bottle
H_2O
H_2O
H_2O
Air
Tracheal
Cannula
2,040 ℓ Plethysmograph
To Pleural or
Esophogeal Cannula
Validyne
MP45 ± 2 cm H_2O
To
Buxco
Amplifier
To
Buxco
Amplifier
Validyne
MP45 ± 50 cm H_2O
Grass
PT5A
Pressure
Transducer
To Grass Physiograph

Figure 2-7. Schematic of the apparatus used to measure lung volumes.

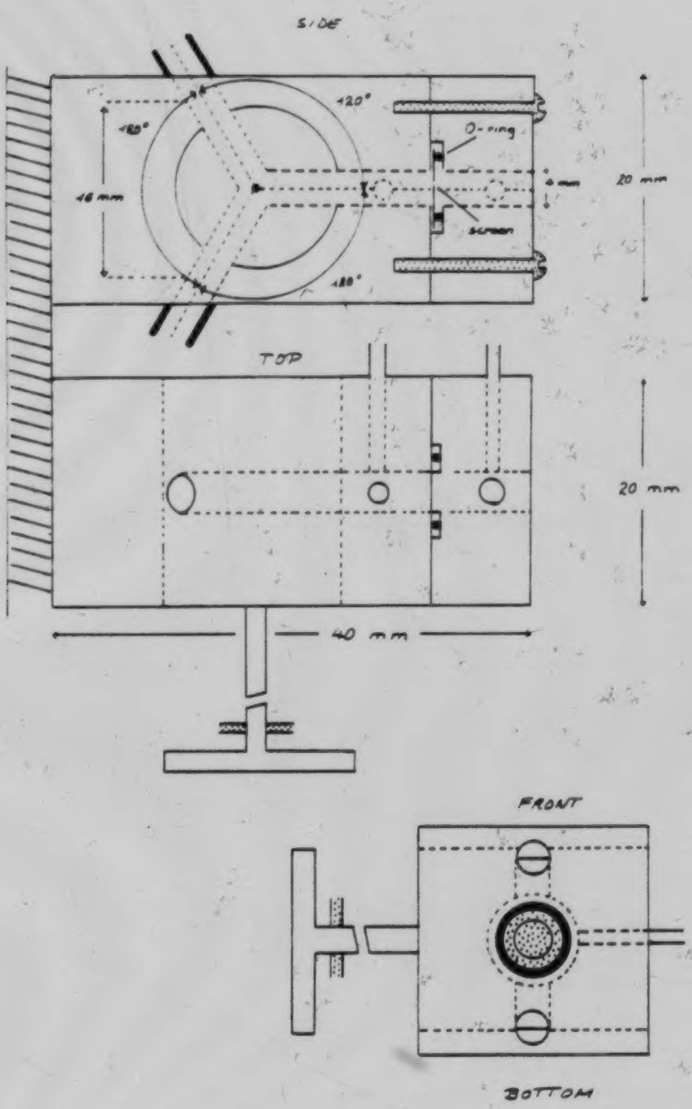
SIDE
120°
120°
120°
16 mm
O-ring
screen
4 mm
20 mm
TOP
20 mm
40 mm
FRONT
BOTTOM

Figure 2-8. Front, side, and top views of the screen pneumotachograph designed by R.L. Jones, and used to measure airflow.

CHAPTER III

ANESTHESIA IN THE GUINEA PIG

Effects of urethane, α-chloralose, and pentobarbital on circulation and respiration.

Introduction

The guinea pig is used extensively as an animal model in biomedical research. Although popular and one of the easiest of animals to handle, guinea pigs are difficult to anesthetize and their response to various anesthetics is unpredictable (101, 102, 120). In this chapter, I report the effects of urethane, α-chloralose, and pentobarbital on MABP, HR, and pulmonary mechanics of normal guinea pigs.

Results and Discussion

Table 3-1 and 3-2 summarize the average baseline values for R_T, E_T, R_L, E_L, V_t, f, MABP, and HR in normal guinea pigs anesthetized with urethane, α-chloralose, or pentobarbital. A compilation of similar data reported in the literature for unanesthetized guinea pigs is presented in Table 3-3.

Del Pozo and Armas (71) have claimed that it is difficult to establish a satisfactory level of anesthesia in guinea pigs when urethane, pentobarbital, or α-chloralose are used. In my experience, a satisfactory level of anesthesia, i.e. absence of toe-pinch (fore and hind) and corneal reflex with regular and stable f, MABP, and HR, was readily attained with urethane (1.25-1.50 g/kg, i.p.). Cardiovascular and respiratory parameters were stable for at least 3 h in freely-respiring and in artificially-ventilated animals. Mean arterial blood pressure and HR were similar in artificially-ventilated and freely-respiring guinea pigs under urethane anesthesia. Thoracic resistance was greater than R_L ($p<0.05$), and E_L was about twice as large as E_T ($p<0.05$) in the freely-respiring and artificially-ventilated guinea pigs under urethane anesthesia(Table 3-1 and 3-2).

Satisfactory levels of anesthesia were not achieved readily in animals using pentobarbital or α-chloralose. Pentobarbital has a narrow therapeutic index in guinea pigs (57, 71, 102). The reaction of guinea pigs to pentobarbital sodium was highly unpredictable. Some animals stopped breathing at doses as low as 25 mg/kg (i.p.), became cyanotic, and then began to respire freely. In other animals, a satisfactory level of anesthesia was achieved only at doses of 40 mg/kg (i.p.). Cannell (57) claims that doses of pentobarbital at doses greater than 40 mg/kg (i.p.) are lethal to guinea pigs, but states that a dose of 37.5 mg/kg (i.p.) provides excellent anesthesia. In my studies, a relatively stable level of anesthesia was established in most animals at doses ranging from 30 to 35 mg/kg (i.p.). At this dose range, f values were relatively low, and V_t high. In the course of an experiment as the effects of pentobarbital began to wane, f rose and V_t fell slightly. This was accompanied by fluctuations in baseline values of R_L, E_L, and MABP, and the restoration of toe-pinch reflexes. Normally, additional injections of pentobarbital (5 mg/kg, i.v.) were given as required to maintain surgical anesthesia.

It was difficult to determine the depth of anesthesia in paralyzed pentobarbital-anesthetized guinea pigs. I assumed that the effects of pentobarbital were waning when baseline MABP fluctuated over 5 mm Hg, at which point an additional injection of pentobarbital (5 mg/kg, i.v.) was administered. Heart rate and MABP of freely respiring and artificially-ventilated guinea pigs under pentobarbital anesthesia did not differ significantly ($p > 0.05$). Pulmonary elastance was greater than

E_T ($p<0.05$) and R_T was greater than R_L ($p<0.05$). The values in the tables are those determined in the first hour following induction of anesthesia (Table 3-1, 3-2).

α-Chloralose was dissolved either in saline heated to 65°C or in 10% polyethylene glycol 200 in saline warmed to 45°C. When given i.p., both solutions caused discomfort to the animal. In addition, the hyperreflexive state induced by the anesthetic made it difficult to perform surgery and to determine if the animal was anesthetized. To overcome these difficulties, I elected to induce anesthesia with methoxyflurane, catheterize a jugular vein, and maintain anesthesia with α-chloralose i.v. Methoxyflurane was chosen for induction of anesthesia because it is easier to administer, has a wider therapeutic index than halothane, and does not irritate the nasal passages or induce the copious nasal secretions associated with ether anesthesia in guinea pigs (36, 102, 120, 263). A major drawback of methoxyflurane is its slow induction (about 4-10 min) and its possible persistence.

α-chloralose administered either i.p. or i.v. following induction with methoxyflurane, depressed respiration. Of the 7 freely-respiring animals, 3 stopped breathing within 1 h of being anesthetized with α-chloralose and were artificially ventilated. I was unable to place an esophageal cannula in these animals and R_L and E_L were not monitored. The V_t and f values listed in Table 3-2 for freely respiring guinea pigs under α-chloralose anesthesia are the levels recorded in these animals within 1 h of induction of anesthesia. In artificially-ventilated animals, baseline values for R_T, E_T, MABP, and HR were stable between injections of test drugs for at least 4 h, and were similar to those reported for the unanesthetized guinea pig (10, 102).

In all three groups of anesthetized guinea pigs, the baseline values for HR did not differ from each other or from those reported for unanesthetized animals (Tables 3-1, 3-2, and 3-3) (101, 102, 120).

Advenier et al. (4) claim that urethane and pentobarbital alter R_L and E_L in guinea pigs. Comparison between R_L and E_L values reported here and those of Advenier and his colleagues is impossible because all my experiments were performed in tracheotomized animals, whereas Advenier used intact animals. Amdur and Mead (10) have reported R_L and E_L values in tracheotomized unanesthetized guinea pigs (Table 3-3). Pulmonary resistances in urethane- and pentobarbital-anesthetized guinea pigs were lower than that reported for tracheotomized unanesthetized guinea pigs (10) ($p<0.05$) (Table 3-2). Although pulmonary elastance in pentobarbital-anesthetized guinea pigs was lower ($p<0.05$) than in unanesthetized guinea pigs, it was unchanged in urethane-treated animals ($p>0.05$).

Of the three anesthetics used, α-chloralose induces the greatest respiratory depression and clearly should not be used in studies requiring freely-respiring animals. In addition, α-chloralose may induce acidosis in some animals. However, this anesthetic had no effect on MABP or HR, and in ventilated animals provided a stable level of anesthesia for at least 4 h (Table 3-1, 3-2).

Pentobarbital reduced f ($p<0.05$) and increased V_t, but $\dot{V}_e$ was still depressed ($p<0.05$). Animals under pentobarbital anesthesia recovered from this initial depression in the course of the experiment. Respiratory rate was a good indicator of the level of anesthesia in these animals. It is difficult to establish a baseline in either freely-respiring or artificially-ventilated animals anesthetized with pentobarbital because of its short duration of action; however, one could attempt a slow i.v. infusion of pentobarbital to maintain a predetermined level of anesthesia as judged by f. Nevertheless, pentobarbital should still be used judiciously in experiments which call for bilateral vagotomy as this procedure induced apnea and death in all of the pentobarbital-anesthetized guinea pigs in which it was attempted.

Urethane depressed f ($p<0.05$), but V_t increased ($p<0.05$), such that $\dot{V}_e$ was greater than that accepted for unanesthetized guinea pigs (Table 3-2, 3-3). In urethane-anesthetized animals, bilateral vagotomy did not produce apnea, but it did reduce f and increased V_t. Thus, urethane was the anaesthetic of choice for the study of the effects of drugs in freely-respiring animals. However, urethane-anesthetized animals stopped breathing when forced to breathe against a closed airway in the manoeuvre used to measure FRC (Chapter IV). Pentobarbital-anesthetized animals had no difficulty with this manoeuvre. Hulbert (personal communication) has also noted this response in urethane-anesthetized animals.

The major drawback of using urethane is the low resting MABP induced by the anaesthetic. Two groups (16, 174) have shown that urethane is a hypotensive agent and an α_2-adrenoceptor antagonist in rats, and have recommended that urethane not be used in experiments involving α-adrenoceptor stimulation. My data clearly show that urethane is a hypotensive agent in guinea pigs. There is no evidence that urethane is an α_2-adrenoceptor antagonist in guinea pigs. Indeed, if it did have α_2-adrenoceptor antagonistic properties, one would expect an elevated baseline MABP (266). However, on the basis of its hypotensive effects, I elected not to urethane in the series of experiments involving α-adrenoceptor stimulation. Neither α-chloralose nor pentobarbital had any effect on MABP. None of the three anesthetics studied affected HR.

In conclusion, one must be very careful in choosing an anesthetic for experiments in guinea pigs. Urethane reduced both vascular and bronchomotor tone in this species, but appears to potentiate $\dot{V}_e$ and may be used if a freely-respiring animal is required. Pentobarbital reduced R_L, E_L, and $\dot{V}_e$; it induced apnea following vagotomy and did not provide a stable baseline in this species. These factors suggest that it should only be used for short-lasting procedures. α-chloralose is a difficult anesthetic to evaluate. It has little effect on MABP and HR in artificially-ventilated animals. Its actions on bronchomotor tone are not known. I was unable to compare the effects of anesthesia on R_T and E_T as there are no reports in the literature on artificially-ventilated, unanesthetized, tracheotomized guinea pigs. However, the baseline R_T and E_T in α-chloralose-anesthetized animals were between those of pentobarbital and urethane groups. This suggests that α-chloralose may also reduce bronchomotor tone. Others have suggested that low bronchomotor tone may be a characteristic of anesthetized guinea pigs (123). The severe respiratory depression induced by α-chloralose clearly mitigates against its use in freely-respiring guinea pigs.

Table 3-1. Mean values (± s.e.m.) of respiratory and cardiovascular parameters in urethane-, pentobarbital-, and α-chloralose-anesthetized guinea pigs. All animals were given decamethonium (10 mg/kg, i.v.) and were artificially ventilated at 0.33 Hz and a V_t adjusted to maintain an end-tidal CO_2 of 4.5 %.

	n	MABP mm Hg	HR beats/min	R_T $cmH_2O/s/ml$	E_T cmH_2O/ml	WEIGHT g
Urethane	10	45±5*	270±10	0.291±0.026¶	1.807±0.079**	400-500
Pentobarbital	13	65±2	247±9	0.398±0.021¶	2.089±0.169**	400-650
α-Chloralose	70	67±2	256±10	0.367±0.018	1.401±0.079	400-700

* $p<0.05$ Significantly less than that reported for unanesthetized guinea pigs (see Table 3-3).

¶ $p<0.05$ Significantly greater than

Table 3-2. Mean values (± s.e.m.) of respiratory and cardiovascular parameters in freely-respiring urethane-, pentobarbital-, and α-chloralose-anesthetized guinea pigs.

	N	MABP mm Hg	HR beats/min	R_L $cmH_2O/s/ml$	E_L cmH_2O/ml	V_t ml	f breath/min	$\dot{V}_e$ ml/min	WEIGHT g
Urethane	13	49±2[*]	299±10	0.266±0.023[*]	3.500±0.328	2.57±0.09[¶]	69±5[*]	176±10[¶]	400-500
Pentobarbital	18	72±3	258±6	0.327±0.033[*]	2.290±0.167[*]	3.25±0.15[¶]	39±2[*]	128±7[*]	400-770
α-Chloralose	7	60±3	257±12	---	---	4.23±0.28[¶]	25±5[*]	105±18[*]	550-650

[*] $p<0.05$ Significantly less than that reported for unanesthetized guinea pigs (see Table 3-3).

[¶] $p<0.05$ Significantly greater than that reported for unanesthetized guinea pigs (see Table 3-3).

Table 3-3: Respiratory and cardiovascular data for unanesthetized guinea pigs at rest (compiled from the literature).

VARIABLE	VALUES
f (breaths/min)	86 ± 13[1]
V_t (ml)	1.7 ± 0.1[1]
$\dot{V}_e$ (ml)	140 ± 5[1]
R_L (cm H_2O/s/ml)	0.38 ± 0.46[1]
E_L (cm H_2O/ml)	4.17*[1]
Systolic Arterial Blood Pressure	90[2]
Diastolic Arterial Blood Pressure	56[2]
MABP	67¶[2]
HR	280 ± 6[2]

[1] AMDUR and MEAD (10)

[2] GREEN (102)

* $E = \frac{1}{C_{dyn}}$

¶ Calculated from systolic and diastolic values.
$\text{MABP} = \frac{1}{3}\,(\text{SYSTOLIC}) + \frac{2}{3}\,(\text{DIASTOLIC})$

CHAPTER IV

Pulmonary resistance and elastance, and airway resistance in the anesthetized guinea pig

Introduction

In pharmacology and in toxicology, guinea pigs are often used as models for the study of airway responses. In such studies, R and E are measured to quantify changes in airway smooth muscle tone. Most studies have used the isolated-points method to determine R and E (10). Pulmonary resistance (R_L) and elastance (E_L) were recorded in freely-respiring animals in this study (Methods pp. 52-55). Thoracic resistance (R_T) and elastance (E_T) were measured in artificially-ventilated animals. A least-squares fit method was used to calculate R_L, E_L, R_T, and E_T in all the experiments. The validity of using this technique to measure R_T and E_T has been discussed by Goel (98). In this chapter, I compare the R_L and E_L values determined by the isolated-points and the least-squares method to verify the validity of the latter technique in pentobarbital-anesthetized guinea pigs. In addition I present R_{av} and FRC values measured in pentobarbital-anesthetized guinea pigs.

Results and Discussion

i) Validity of R_L and E_L measurements

The validity of the R_L and E_L measurements depends on the reliability of the P_{es}, $\dot{V}$, and V signals. Esophageal pressure was used to estimate P_{pl}. In 5 animals, P_{es} and P_{pl} were recorded simultaneously and displayed on an oscilloscope. The tracings had an identical baseline and amplitude, and were in phase at 0.33 Hz in the 5 animals studied. Airflow was determined via a pneumotachograph connected to a differential pressure transducer. The response of the system was linear over the range (0 - 15 ml/s) of flows tested. Volume was measured by plethysmography for the isolated-point method and by integrating the $\dot{V}$ signal for least-squares. The V_t measured by integration and plethysmography were equal. Integration has some advantages over plethysmography. The latter requires a sealed box and isothermal conditions. This is not easily accomplished and even the best designed boxes show some leakage which must be minimized. To maintain isothermal conditions, I chose to keep the box at 37°C by pumping the heated water through an external jacket. Initially, this did not work and the P_{box} signal drifted over the course of the experiments. I assumed that the drift was due to the inability of the pump/heater to adjust to added heat generated by the animal. Connecting a 1L reservoir to the box via a low resistance connector helped to stabilize the temperature, presumably by acting as a "heat sink".

It is also important to ensure that the three signals are in phase in order to avoid errors in the measurement of R_L and E_L due to phase lags. The response characteristics of the transducers used to determine V, P_{es}, and $\dot{V}$, were not evaluated directly. However, P_{es}, $\dot{V}$, and V signals were monitored on an oscilloscope before measuring R_L and E_L and no phase shifts were observed.

Table 4-1 lists the mean R_L and E_L values calculated manually using the isolated-points method and by a computer using a least-squares fit. Pulmonary resistance and E_L were determined breath-by-breath over an identical period to ensure that the same V, $\dot{V}$, and P signals were used for both methods. The findings show that the two methods yield equivalent values of R_L and E_L consistent with those reported in the literature (10, 118, 231).

Although there are now good digital and analog computer systems that use the isolated-points method to calculate R_L and C_L (231), the computer-assisted least-squares fit offers several advantages. Best-fit estimators are the most accurate means of fitting constants to an equation because they minimize the summed squared error between observed points and the best-fit curve. Moreover, unlike the isolated-point technique which focuses on given points in a breath cycle, the least-squares fit uses an array of points throughout the cycle. Thus, the least-squares technique is not subject to the errors associated with determining zero $\dot{V}$ (66).

The least-squares technique has some limitations. This method does not separate the P signal into "pure" flow-resistive and volume elastic components - therefore the changes in R_L and E_L are interdependent on each other. However, Goel (98) has shown the system is sensitive to independent changes in R_T and E_T. In most of my experiments, changes in R_L and E_L occurred simultaneously, but in some experiments either R_L or E_L was altered independently of the other.

As mentioned in the Chapter III, R_T was greater than R_L in pentobarbital- and urethane-anesthetized guinea pigs; whereas, E_L was twice as large as E_T in urethane-anesthetized guinea pigs, but did not differ from E_T in pentobarbital-anesthetized guinea pigs. From the equations relating thoracic to pulmonary parameters, one would predict that R_T and E_T should be greater than R_L and E_L. This suggests that the measurements of R_L and E_L are invalid. However, it should be noted that the thoracic and pulmonary parameters were measured in different sets of animals. Thoracic and pulmonary measurements do provide an index of changes in airway caliber; however, they were not monitored simultaneously and are not comparable. Thoracic resistance and E_T were measured only during inspiration, whereas R_L and E_L over the entire breath. Pulmonary resistance and E_L vary with depth and the frequency of respiration. In freely-respiring animals, depth and frequency are dependent on type of anesthetic used and the level of anesthesia established, and vary greatly among animals (Chapter III). In ventilated animals, the depth (per kg b.w.) and the frequency of ventilation were independent variables and were constant for all animals studied. The ventilated animals are also paralyzed and the effects of the inspiratory muscles are excluded. Freely-respiring animals breathe at a fast rate. This can lead to time constant inequalities in the filling of different parts of the lung, an increased viskance component, and hyperinflation. All these can in turn affect R_L and E_L measurements. In the artificially ventilated animal, f was low. This minimizes time constant inequalities, reduces viskance, and should allow the animal to exhale completely before the next breath.

ii) Functional residual capacity

In the course of measuring R_{aw} using a plethysmograph, FRC is measured to determine the relationship between P_{ao} and P_{box} (82). Table 4-2 summarizes the absolute FRC and FRC/kg b.w. of 6 female pentobarbital-anesthetized guinea pigs, and a list of similar data for this species compiled from the literature. It should be noted that the FRC values attributed to Takazawa et al. (247) are predicted and not actual data. This group measured FRC in 12 male guinea pigs ranging in weight from 250-1000 g using the gas dilution method, and determined that log FRC was linearly related to log b.w. by the following equation:

$$\log \text{FRC (ml)} = -0.26 + 0.42 \log \text{b.w. (g)} \qquad \text{(Eqn. 4-1)}$$

I used their equation to predict the FRC for 6 animals in my sample group.

The data in Table 4-2 show that the FRC/kg b.w. for the unanesthetized guinea pig reported by Raub and Gillespie (205), is much greater than that observed in my sample of 6 female pentobarbital-anesthetized guinea-pigs, and that reported by others (66, 247). This suggests - as Raub and Gillespie (205) have claimed - that pentobarbital reduces FRC in this species. Some anesthetics are reputed to decrease FRC in man (207), however, there is some question as to the validity of data of Raub and Gillespie (205): 1) The FRC/kg b.w. values they report for unanesthetized guinea pig are approximately three times greater than those reported for unanesthetized rats (14.4 ml/kg) (135); 2) These data have appeared only in an abstract and have been cited in a review by one of the authors (97); 3) The method used to measure FRC was not reported; 4) Their data remain unconfirmed.

The FRC/kg b.w. of my small sample of guinea pigs is much less than that predicted by Takazawa et al. (247), but within the range observed by Crosfill and Widdicombe (66). It is difficult to explain the differences among the studies. All animals were anesthetized with pentobarbital, however different methods were used to measure FRC. Crosfill and Widdicombe (66) measured FRC by excising the lungs and determining the gas volume by immersing the lungs in saline. This is the only absolute measure of FRC, but, as they note, it may underestimate FRC slightly due to the absorption of gas under the elastic tension of the lungs. One would expect that the gas-dilution method used by Takazawa et al. (247) would also underestimate FRC (in comparison with the plethysmographic method I used) because gas-dilution excludes the volume of non-ventilated gas (82). However, Takazawa and his colleagues used only male guinea pigs, while I used females exclusively. Crosfill and Widdicombe (66) did not report the sex of their animals. Functional residual capacity has been reported to be lower in women than in men (91), but the difference is not as great as the discrepancy between the Takazawa's predicted FRC for male guinea pigs and that which I observed in females.

iii) Airway resistance, pulmonary resistance, and specific airway conductance

Airway resistance was measured in 4 animals using the method of Dubois et al. (83) and sG_{aw} was calculated from the R_{aw} and FRC. Airway resistance was compared with R_L measured by least-squares fit in the same animals. The data are summarized in Table 4-3. Airway resistance was about 85% of R_L. In man, R_{aw} is about 80% of R_L (91).

This is the first report of R_{aw} in guinea pigs. Mitchell (173) claims to have measured R_{aw}, but in fact calculated R_L using the isolated-points method. Agrawal (7) determined sG_{aw} directly in unanesthetized guinea pigs. The mean sG_{aw} of the 4 pentobarbital-anesthetized guinea pigs in this study was significantly ($p < 0.05$) greater than that observed by Agrawal in 42 anesthetized guinea pigs. This discrepancy reflects the fact that my studies were conducted in tracheotomized guinea pigs and Agrawal measured sG_{aw} in intact guinea pigs. The upper respiratory tract in guinea pigs is estimated to account for about 45% of the total R_L (10). Agrawal (7) did not measure R_L or R_{aw} in his study, but he assumed that R_L would be equal to that measured by Amdur and Mead in intact unanesthetized guinea pigs.

Table 4-1: Pulmonary resistance (R_L) and dynamic pulmonary elastance (E_L) determined manually by the isolated-points method and by computer using a least-squares fit in 5 normal freely-respiring guinea pigs.

METHOD	R_L cm H_2O/s/ml	E_L cm H_2O/ml
Isolated points	0.28 ± 0.05	2.67 ± 0.21
Least-squares	0.26 ± 0.02	3.06 ± 0.30

Table 4-2: Functional residual capacity of 6 pentobarbital-anesthetized female guinea pigs and a compilation of FRC reported for guinea pigs.

	n	Sex	FRC ml	FRC/kg b.w. ml/kg	Anesthetic
Present study	6	F	4.49 ± 0.30	9.85 ± 0.83	Pentobarbital
Takezawa *et al.*, (247)	12	M	7.2	15.9	Pentobarbital
Raub & Gillespie (205)	36	M	13.7	44.3	None
Crosfill & Widdicombe (97)	-	-	4.5	6.9	Pentobarbital

Table 4-3: Airway resistance and specific airway conductance in 4 pentobarbital-anesthetized guinea pigs.

	R_{aw} cm H_2O s ml^{-1}	sG_{aw} cm H_2O^{-1} s^{-1}
Present study	0.261 ± 0.041	0.843 ± 0.098
Agrawal (1981)	-----	0.48

CHAPTER V

Effects of Non-steroidal-anti-inflammatory Drugs and Tartrazine on Carotid-Sinus-Nerve Activity and Arterial Blood Pressure in Guinea Pigs.*

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* A version of this chapter has been published in Pharmacology (1987) 34(2-3): 96-103.

Introduction

In susceptible patients, ASA induces characteristic bronchospasm and rhinitis, a reaction often referred to as ASA-idiosyncrasy (239, 243). Many patients sensitive to ASA react similarly to other NSAID and to the coloring agent TZ (219, 239, 243).

In guinea pigs, ASA, indomethacin (IND) and TZ, (i.v.) induce reflex bronchoconstriction which is blocked by sodium cromoglycate (SCG), catecholamine depletors, and bilateral glossopharyngealectomy, but is unaltered after bilateral vagotomy or atropine administration (41). An apparently identical response can be induced by orthodromic electrical stimulation of CSN in guinea pigs, and this can be differentiated pharmacologically into two components: one is blocked by SCG and mepyramine, and the other by atropine (44). These findings suggest that NSAID and TZ act via CSN afferents and that SCG may act on either the afferent or efferent side of the reflex arc of drug-induced bronchoconstriction (44).

I report here the effects of ASA, IND, and TZ on MABP and spontaneous activity in the CSN, together with the effects of SCG and phentolamine on these responses in guinea pigs.

Results

i) Anatomy of the carotid sinus and its innervation

The anatomy of the carotid sinus and its innervation varied among the 80 guinea pigs used in this series of experiments. Of the 80 animals, 16 did not have an anatomically distinct sinus or CSN on either side, while 27 were asymmetrical with a defined sinus and CSN on only one side. In 32 of the 37 animals with bilaterally defined sinuses and CSN, the nerve emerged from the sinus apposed to the APA or the OA and merged with the IX nerve at the level of the jugular foramen. In the remaining 5 animals, the CSN joined the superior laryngeal nerve as it traversed the carotid bifurcation. These CSN were quite short and it was extremely difficult to record from them. All defined sinuses showed sympathetic innervation emanating from the superior cervical ganglion located immediately dorsal to the sinus.

ii) Baseline (Control) Values

In the guinea pigs studied, MABP was 40 ± 2 mm Hg. Heart rates ranged from 240 to 300 beats/min. Control neurograms from CSN showed bursts of activity synchronous with the systolic pulse (Fig. 5-1). Asynchronous activity was rarely seen and, when present, was never prolonged. Carotid-sinus-nerve activity and MABP returned to baseline values between injections throughout the 3-h experimental period.

iii) Effects of ASA, IND, and TZ

Acetylsalicylic acid (0.1-1.0 mg/kg), IND (0.1-1.0 mg/kg) and TZ (0.1-2.0 mg/kg) i.v. increased both CSN activity and MABP without altering HR (Tables 5-1 - 5-3). Increases in CSN activity and MABP were maximal within 30 s of injection and returned to baseline in 60 s. At higher doses (> 0.5 mg/kg) of all drugs, MABP and CSN activity fluctuated for 5 min following the initial response, and breathing appeared labored in all animals. Increases in CSN activity induced by all three drugs were confined to increases in activity synchronous with systole (Fig. 5-1). Increases in CSN activity induced by the drugs varied greatly among animals. Comparison of the mean CSN activity responses to determine dose-dependency indicated that the actions of ASA, IND, and TZ were not dose-related. However, responses appeared to be dose-dependent within animals (Table 5-1 to 5-3). When the data were examined by ANOVA and coefficient of correlation, ASA, IND, and TZ were shown to induce dose-dependent increases in CSN activity (Table 5-5), although the dose-response lines were not steep. Indomethacin was the most effective agent and TZ the least. Increases in MABP induced by ASA, IND and TZ showed less interanimal variation; however, only the increases induced by IND were dose-dependent. The increases in CSN activity induced by ASA, IND, and TZ did not correlate with increases in MABP (Table 5-5).

Sodium salicylate (0.1-1.0 mg/kg, i.v.) did not alter either MABP or CSN activity.

iv) Effects of Phenylephrine

Phenylephrine (PE) (2.0-10.0 μg/kg, i.v.) induced dose-related increases in both CSN activity and MABP. Increases in CSN-activity were correlated with increases in MABP (Tables 5-4, 5-5).

v) Effects of SCG

Sodium cromoglycate (10.0 mg/kg, i.v.) increased CSN activity (17 ± 9%, n=12) and MABP (41 ± 2 to 46 ± 4 mm Hg), but neither effect was statistically significant. Comparison of the mean responses to ASA, IND, and TZ (1.0 mg/kg) and PE (10.0 μg/kg) administered before and after SCG, indicated no significant effects of SCG on drug-induced increases in MABP and CSN activity (Table 5-6). However, within animals, SCG consistently reduced the effects of ASA and IND on CSN activity, and comparison of the differences revealed that the reductions were statistically significant (mean reduction 16 ± 1% for IND and 12 ± 2% for ASA; $p<0.05$). In three of four guinea pigs, SCG also reduced CSN increases induced by TZ (1.0 mg/kg). Sodium cromoglycate did not affect the MABP increases induced by ASA, IND, and TZ, or the MABP and CSN responses to phenylephrine (Table 5-6).

vi) Effects of phentolamine

Phentolamine (0.2 mg/kg) reduced MABP (43 ± 3 to 24 ± 3 mm Hg, n=4) and induced asynchronous activity that lasted about 1 min and recurred intermittently for up to 1 h. Phentolamine inhibited increases in CSN activity induced by ASA, IND, TZ and phenylephrine (Fig. 5-2). It was found to have no statistically significant effect on the increases in MABP induced by ASA, IND, and TZ when these drug-induced changes were expressed as a percentage of control. By contrast, when the data were expressed in absolute terms, phentolamine was shown to reduce ASA- and TZ-induced increases in MABP without altering the response to IND (Fig. 5-3). Phenylephrine-induced increases in MABP were significantly reduced by phentolamine, irrespective of the manner in which the data were expressed.

Discussion

I report here the first successful recordings of spontaneous nerve impulses from intact CSN in guinea pigs. In this species, the CSN are quite fine and, in many cases, poorly defined, making it difficult to separate the nerve into individual fibers. Carotid-sinus nerves contain two types of receptor afferents: baroreceptor afferents, which respond to elevations in intrasinus blood pressure, and chemoreceptor afferents, which respond to changes in blood PO_2, PCO_2, and pH. Carotid-sinus-nerve-afferent discharges in bursts synchronous with heart rate were identified as baroreceptor activity, whereas asynchronous impulses having no apparent relation to systole were identified as chemoreceptor activity (117).

All drug-induced changes in CSN activity and MABP reported in this study were expressed as a percentage of control. The advantage of expressing data in this manner is that it standardizes changes between animals. The disadvantage is that the degree of change may be obscured or magnified depending on the baseline. Changes in CSN activity are expressed as a percentage of control because absolute data, as determined by integration, are dependent on the number of fibers on the electrodes, the gain of the system, the signal-to-noise ratio, and vary greatly between preparations. Thus, absolute changes in CSN activity are meaningless numbers without a point of reference. Increases in MABP were expressed as a percentage of control to standardize the responses. It is unlikely that changes in MABP have been obscured or magnified between preparations, as baseline MABP did not vary greatly among urethane-anesthetized animals. However, phentolamine reduced baseline MABP; in doing so, it may have obscured its effects on the pressor actions of the NSAID and TZ.

In the present study, ASA, IND, and TZ (i.v.) induced dose-dependent increases in CSN activity. All three agents increased MABP, but only the IND-induced increases were dose-dependent. MABP increases induced by ASA and TZ were not artifacts, as equal volumes of isotonic saline did not elicit similar responses. The lack of dose-dependency in the MABP response to ~~ASA~~ and TZ is puzzling, but not novel. Prostaglandin-induced bradycardia and hypotension has also been shown to be dose independent (119). It has been pointed out that the CSN activity responses to ASA, TZ, and IND varied greatly between animals and that the dose-response lines were not steep. Although this could suggest that the doses tested were at either extreme of the dose-response curves, this is unlikely. In examining the bronchoconstrictor actions of these agents, a full dose-response curve was generated (41). The doses tested in the present study were within the linear range of this dose-response curve. It is significant that the dose-response curves reported in the earlier study also showed high variability and were not steep. The variation between animals and the flat dose-response lines may reflect the nature of the response to these agents.

It is difficult to separate the pressor effects of the NSAID and TZ from any direct action on CSN afferents. I attempted to separate these two responses by comparing the relationship between increases induced by ASA, IND, and TZ, and the α-adrenoceptor agonist phenylephrine. Increases in CSN activity and MABP to phenylephrine were correlated, whereas those to ASA, IND, and TZ were not. This suggests, but does not demonstrate, that ASA, IND, and TZ may act directly on CSN afferents.

The lack of asynchronous activity in response to these three agents implies that their effects on CSN afferents are confined to CBr. This conclusion is presumptive, as I was unable to successfully record from either homogenous CBr or CCr bundles, or single receptor units. Selective stimulation of both CCr and CBr fibers have been shown to elicit bronchoconstriction (68, 182).

The possible role of PG in the actions of ASA and IND on CSN activity and MABP has not been explored in the present study. In addition, the doses of ASA and IND were chosen on the basis of their ability to induce bronchoconstriction, rather than their effects on PG synthesis. Stimulation of PG synthesis induces hypotension and bradycardia, and it has also been reported to modulate arterial baroreflexes indirectly by via cardiopulmonary C-fibers located in the left ventricle (119). Inhibition of PG synthesis may account for the actions of ASA and IND in guinea pigs; but, the rapidity of onset of the increases in CSN-activity and MABP, while not excluding PG involvement, does reduce its likelihood (188). In humans, NSAID which precipitate bronchospasm have anti-cyclooxygenase activity; whereas, those lacking anti-cyclooxygenase activity are not bronchospastic (243, 244). Tartrazine does not inhibit cyclooxygenase; thus, its effects may have little to do with PG synthesis (243). However, TZ sensitivity is a rarer phenomenon than was believed initially, and it would appear that the anti-cyclooxygenase activity of NSAID is significant in sensitive asthmatics.

In guinea pigs, SCG blocked the bronchoconstrictor actions of ASA, IND, and TZ, but only reduced the bronchoconstriction induced by orthodromic electrical stimulation of the CSN (41). Thus, it was suggested that SCG antagonized the effects of ASA, TZ, and IND on CSN afferents, and inhibited their reflex actions on the airways. In the present study, SCG at a dose previously shown to block drug-induced bronchoconstriction (10 mg/kg, i.v.), decreased the actions of ASA, TZ, and IND on CSN activity. Generally, reports of SCG's actions on afferents are inconsistent. Dixon et al. (74) demonstrated that SCG blocked capsaicin-induced increases in C-fiber activity in dogs, but others (65) could not confirm this. The selective reduction by SCG of ASA- and IND-induced increases in CSN activity accords with the mechanism of action proposed by Biggs (41); however, the small size of the reductions indicates that CSN afferents are not the principal site for blockade by SCG of the bronchoconstrictive actions of NSAID and TZ. Although SCG has been reported to reduce MABP in dogs (131), we observed no hypotensive effects of this agent in guinea pigs.

Phentolamine reduced the increases in CSN activity and MABP induced by ASA and TZ. It also decreased the actions of IND on CSN activity without affecting the MABP response. These differential effects are puzzling. They imply that the increases in CSN activity induced by ASA and TZ were due to their effects on the vasculature, whereas IND apparently had separate actions on CSN activity and MABP. However, this is speculative. The relationship between the MABP and CSN activity effects of these drugs have not been differentiated in the present study. In order to determine whether these agents increase CSN activity by a direct action or by their actions on the vasculature, their effects on CSN activity should be examined using a vascularly isolated carotid sinus preparation in which ISP may be controlled independently of the systemic blood pressure.

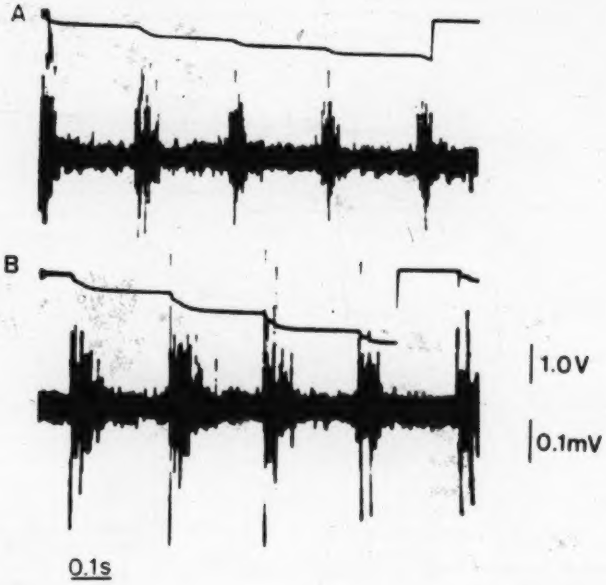

Figure 5-1. Oscilloscope tracings of integrated (top trace) and unintegrated (bottom trace) spontaneous CSN activity in urethane-anesthetized guinea pigs: a) control (before administration of drugs); b) 30 s after injection of tartrazine (1.0 mg/kg). (The tracing of the integrated CSN activity is inverted).

TABLE 5-1. Effects of ASA on MABP and CSN activity in 4 guinea pigs.

Dose mg/kg	Parameter	RESPONSE (Increase as % control) 1	2	3	4	Mean ± SE
0.1	CSN activity	35	0	24	16	19 ± 7
	MABP	9	0	6	9	6 ± 2
0.5	CSN activity	47	19	44	35	36 ± 6
	MABP	7	0	30	8	11 ± 7
1.0	CSN activity	72	52	40	45	53 ± 7
	MABP	16	11	22	20	17 ± 3

Table 5-2. Effects of IND on MABP and CSN activity in 4 guinea pigs.

Dose mg/kg	Parameter	RESPONSE (Increase as % control) 1	2	3	4	Mean ± SE
0.1	CSN activity	31	0	70	17	30 ± 15
	MABP	4	0	0	4	2 ± 1
0.5	CSN activity	72	40	51	44	52 ± 7
	MABP	37	10	12	23	21 ± 6
1.0	CSN activity	96	87	128	105	104 ± 9
	MABP	24	40	20	25	23 ± 8

Table 5-3. Effects of TZ on MABP and CSN activity in 4 guinea pigs

Dose	Parameter	RESPONSE (Increase as % control)				
mg/kg		1	2	3	4	Mean ± SE
0.1	CSN activity	25	6	35	8	19 ± 7
	MABP	38	13	9	10	18 ± 7
0.5	CSN activity	34	32	50	20	34 ± 6
	MABP	20	35	9	20	21 ± 5
1.0	CSN activity	41	40	56	25	41 ± 6
	MABP	24	40	20	25	27 ± 8
2.0	CSN activity	28	60	49	27	41 ± 8
	MABP	20	22	23	27	23 ± 2

Table 5-4. Effects of phenylephrine on MABP and CSN activity in 4 guinea pigs.

Dose	Parameter	RESPONSE (Increase as % control)				
μg/kg		1	2	3	4	Mean ± SE
2.0	CSN activity	40	53	23	67	46 ± 9
	MABP	33	33	29	25	30 ± 2
4.0	CSN activity	86	77	60	-	74 ± 8
	MABP	61	62	56	-	60 ± 2
10.0	CSN activity	93	136	140	137	127 ± 11
	MABP	90	100	85	93	92 ± 3

Table 5-5. Summary of statistical analyses.

Drug	N	Parameters	Correlation Coefficient	Line Slope (least-squares analysis)	F (ANOVA)
Aspirin					
	12	Log dose vs CSN activity[1]	0.74*	32.0	13.2*
	12	Log dose vs MABP[1]	0.52	10.6	3.0
	12	CSN activity[1] vs MABP[1]	0.50	1.3	-
Indomethacin					
	12	Log dose vs CSN activity[1]	0.80*	74.9	23.8
	12	Log dose vs MABP[1]	0.75*	22.9	>50*
	12	CSN activity[1] vs MABP[1]	0.49	1.4	-
Tartrazine					
	16	Log dose vs CSN activity[1]	0.60*	18.4	5.1*
	16	Log dose vs MABP[1]	0.24	4.9	0.8
	16	CSN activity[1] vs MABP[1]	0.10	0.2	-
Phenylephrine					
	12	Log dose vs CSN activity[1]	0.85*	90.0	>50*
	12	Log dose vs MABP[1]	0.98*	70.0	>50*
	12	CSN activity[1] vs MABP[1]	0.86*	1.2	-

[1] Percentage increases from control values in individual animals.

* Statistically significant ($p<0.05$).

Table 5-6. Effects of SCG on the increases in MABP and CSN activity induced by ASA, IND, TZ and phenylephrine in 4 guinea pigs.

Drug and dose	Parameter	Response (percentage increase above control) 1	2	3	4	Mean ± SE
ASA 1.0 mg/kg	CSN activity	41	37	21	25	31 ± 5
	MABP	10	28	14	21	18 ± 4
ASA 1.0 mg/kg + SCG 10.0 mg/kg	CSN activity	39	15	8	15	19 ± 7
	MABP	8	30	14	21	18 ± 5
IND 1.0 mg/kg	CSN activity	30	110	55	56	62 ± 17
	MABP	38	75	18	50	45 ± 12
IND 1.0 mg/kg + SCG 10.0 mg/kg	CSN activity	16	90	41	38	46 ± 16
	MABP	35	30	40	35	35 ± 2
TZ 1.0 mg/kg	CSN activity	40	77	35	39	46 ± 10
	MABP	25	50	50	20	38 ± 9
TZ 1.0 mg/kg + SCG 10.0 mg/kg	CSN activity	32	32	50	20	34 ± 11
	MABP	25	50	50	20	36 ± 8
Phenylephrine 10 μg/kg	CSN activity	93	136	80	150	115 ± 17
	MABP	88	100	84	93	91 ± 4
Phenylephrine 10 μg/kg + SCG 10.0 mg/kg	CSN activity	100	142	84	153	120 ± 17
	MABP	85	100	50	75	78 ± 11

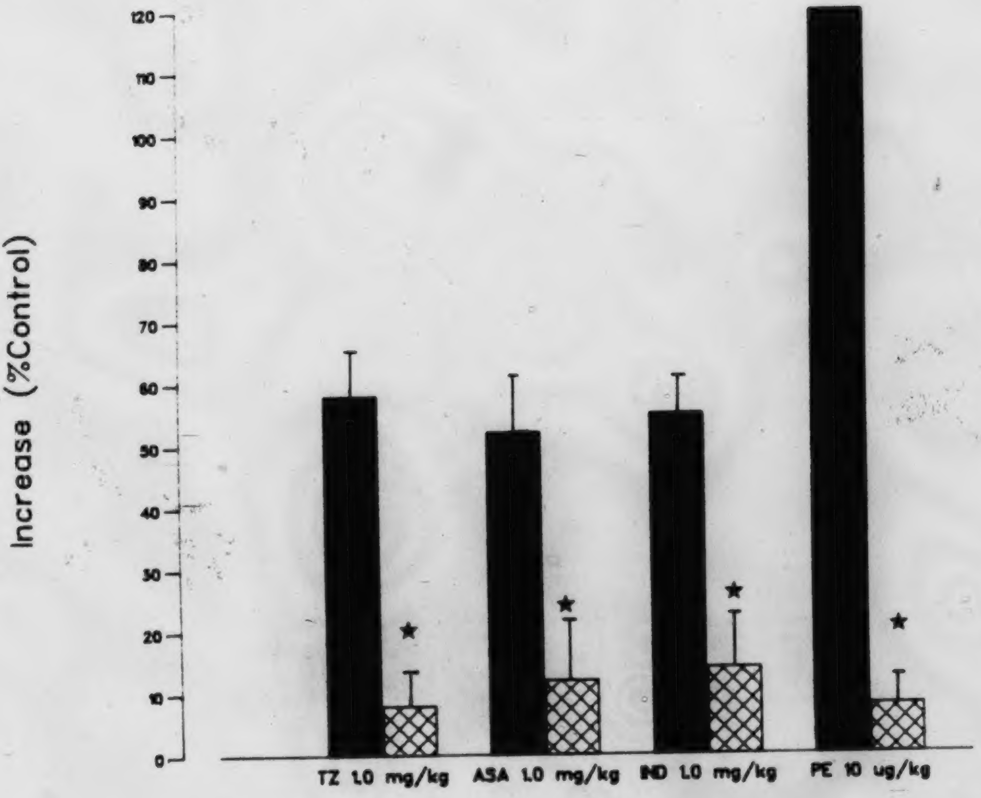

Figure 5-2. Effects of phentolamine (0.2 mg/kg) on increases in CSN-activity induced by ASA, IND, TZ, and phenylephrine. Each bar represents the mean maximal increase ± s.e.m. in 4 guinea pigs, solid bars before, and the hatched bars after phentolamine. ★$p < 0.05$.

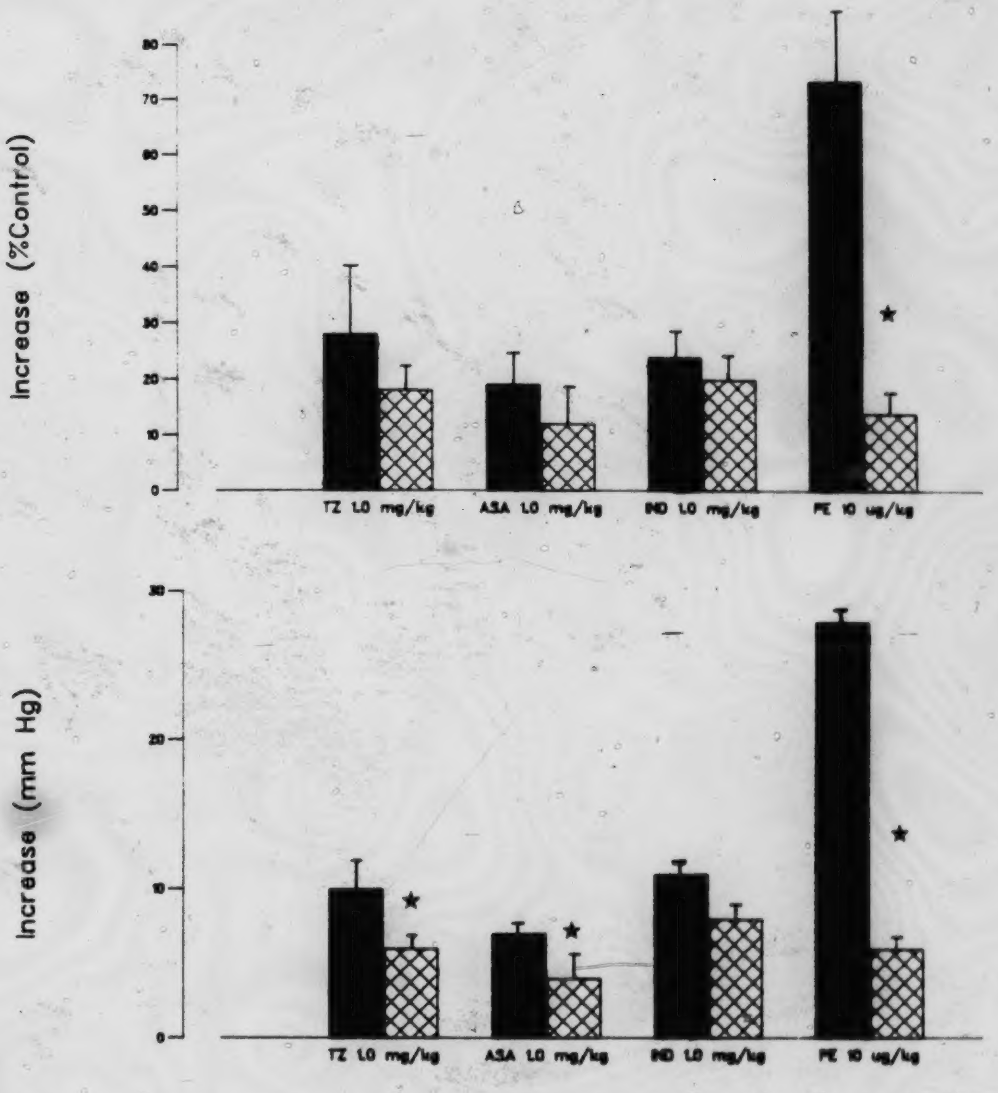

Figure 5-3. Effects of phentolamine (0.2 mg/kg) on increases in MABP induced by ASA, IND, TZ, and phenylephrine. a) Changes are expressed as a percentage of control; b) Actual changes are given in mm Hg. Each bar represents the mean maximal increase ± s.e.m. in 4 guinea pigs, solid bars before, and the hatched bars after phentolamine ★$p < 0.05$.

CHAPTER VI

Effects of ASA and TZ on CSN activity in the isolated carotid sinus

* A version of this chapter was presented at the Federation of American Societies of Experimental Biology April 1986 Meeting in St. Louis. An abstract of this material appears in Federation Proceedings (1986) 45: 204.

Introduction

Carotid baroreceptors respond to the deformation of the walls of the carotid sinus induced by increases in arterial blood pressure (145). The response of CBr to increases in blood pressure is influenced by various agents including NA (126, 253, 276), calcium antagonists (111), and VP (126). I showed that NSAID and TZ administered i.v. induce dose-dependent increases in CSN activity, accompanied by an increase in MABP (Chapter V). The increases in CSN activity were associated with an increase in CBr activity and were independent of the accompanying rise in MABP. This led me to propose that NSAID and TZ increase CSN activity by a direct action on CBr. In the study described below, I attempted to confirm that NSAID and TZ act directly on CBr by developing a perfused, vascularly-isolated carotid sinus preparation to assess the effects of the drugs on CBr independently of their actions on the vasculature.

Results and Discussion

Isolation of the sinus

The CSN was first located and identified by its characteristic discharge in synchrony with systole. A CSN was identified in 28 of 32 guinea pigs used in this study and the sinus was isolated vascularly and perfused successfully in 14 of the 28 animals.

After isolating the sinus, the CSN and the vagal and sympathetic nerve supplies were sectioned distally to remove efferent input to the sinus. The CSN was then placed on bipolar electrodes. CSN neurograms showed continuous random low-level discharge when the sinus was perfused below the CBr threshold (see later). I assumed that the low-level activity seen below the CBr threshold was due to CCr discharge because it remained constant when ISP was lowered to 0 mm Hg. In other species, CCr discharge was reported when ISP was maintained below CBr threshold (111). The presence of CCr fibers in the CSN was evidenced by a large increase in CSN activity when NaCN (20 μg/kg, 0.01 ml) was injected retrogradely into the sinus.

I tried to record from single receptor units or homogenous bundles of CCr and CBr fibers in 2 animals. The CSN in guinea pigs is very fine and, although I was able to divide the nerve, I could not record successfully from the twigs. I decided not to pursue single fiber recordings due to the technical difficulties and the low success ratio of the vascularly-isolated sinus preparation.

In 2 animals, I attempted to desensitize CCr selectively by local injection of acetic acid into the sinus (96). Retrograde injection of acetic acid (0.05 ml 0.15 N) into the sinus induced a large increase in CSN activity which was similar to that induced by NaCN. This increase in activity subsided within 2 min of the injection of acetic acid and the low-level "CCr activity" was no longer present below the CBR threshold. However, acetic acid was toxic to CBr - increases in ISP failed to induce CSN activity - even 1 h after injection of acetic acid.

Because I was unable to separate CCr and CBr fibers surgically and chemically, I elected to study the effects of NSAID and TZ on whole-nerve CSN activity. In order to differentiate between CCr and CBr discharge, I assumed that any increase in CSN activity that resulted from an increase in ISP was due solely to CBr discharge. To generate an ISP-CSN activity curve, ISP was first reduced to 0 mm Hg and then slowly raised until an increase in CSN activity was noted. This point was defined as the CBr threshold. Intrasinus pressure was then increased stepwise in 10-mm Hg increments. Carotid-sinus-nerve activity increased and rapidly (<30 s) adapted to a new steady state level after each pressure step. This response was similar to that reported in single unit CBr preparations (92). The steady-state level of CSN activity at a given ISP was used to study the relationship between ISP and CSN activity. Each ISP-CSN activity curve was generated in about 5 min.

Control Curves

Figure 6-1 shows the curve relating CSN activity to ISP in the absence of drugs (Krebs solution only, control). The threshold and saturation pressures, and the slope of the linear portion of the ISP-CSN activity curve are summarized in Table 6-1. Slope was used to assess CBr sensitivity.

Whole-nerve CSN recordings indicated that guinea-pig CBr have lower thresholds and saturation pressures than those reported for single CBr units in adult and newborn rabbits (254), monkeys (253), dogs (232), and cats (216). The sensitivity of guinea-pig CBr - perhaps reflecting their lower operational pressure range - is greater than that determined in dogs from whole nerve or multifiber recordings (111). Their lower threshold and operational pressures, and their greater sensitivity, imply that guinea-pig CBr have a lower "setpoint" than CBr in other species, and may account for the low resting MABP seen in the former species. The resting MABP in the monkey, the dog, the rabbit, and the cat is higher ($\geq$ 30 mm Hg) than that reported in unanesthetized guinea pigs (101). The underlying mechanism for this low setpoint - or "setpoints" in general - is unknown. Guinea-pig CBr and carotid sinus are histologically similar to those in other species (228). This homogeneity among species suggests that the differences in CBr sensitivity cannot be explained on a structural basis.

Sources of Error

There are a number of problems inherent in whole nerve recordings and in the assumptions I made to differentiate between CCr and CBr. Firstly, while I assumed that all CSN activity that resulted from an increase in ISP was due to CBr discharge, CCr activity was not eliminated. Carotid chemoreceptor discharge varies inversely with sudden changes in ISP (46). If this variation occurred during the generation of ISP-CSN activity curve, it may have led to an underestimate of the dynamic response, however this should not affect the steady-state CSN activity which was used as an index of CBr activity. Also, I assumed that the sinus would remain well-oxygenated throughout the generation of an ISP-CSN activity curve. If this did not hold true, then one would expect that CSN activity below the CBr threshold should increase as CCr fibers discharge in response to the hypoxia. I examined this by leaving the sinus unperfused for 5 min - the maximum period of time required to generate a ISP-CSN activity curve - and found that CCr activity did not increase appreciably. In addition, at least 2 control curves were generated in order to eliminate any differences in CCr activity. The experiment was undertaken only when the curves were within 5% of each other. Thus, I was sure that increases in CSN activity resulting from the step increments in ISP were due solely to CBr.

Secondly, the absolute value of nerve activity in whole nerve recordings will vary with the number of fibers on the electrodes (111). I compensated for any differences in the absolute values among animals by expressing CSN activity as a percentage of maximum control activity.

Thirdly, CBr are innervated by both myelinated and unmyelinated afferents. Generally, potentials from unmyelinated afferents have a smaller amplitude than those from myelinated afferents. In addition, CBr served by unmyelinated fibers have a higher threshold pressure than those served by myelinated afferents (53). It may be argued that whole nerve recording and the integration technique I used to quantify CSN activity were skewed in favor of large amplitude discharge. This may account in part for the low CBr 'setpoint'. However, amplitude depends not only on the fiber type, but on the inter-electrode resistance and the distance of the fiber from the electrode surface. The ratio of unmyelinated to myelinated fibers in the guinea pig ranges from 13:1 to 18:1 (228). The number of unmyelinated fibers on the electrodes greatly exceeds the number of myelinated fibers, thus it is unlikely that unmyelinated discharge was ignored in this study. Clearly, this is speculative on my part as I have no direct evidence of the presence of unmyelinated and myelinated activity. Given the length, the fineness, and the location of the nerve, I was unable to differentiate between myelinated and unmyelinated activity.

Finally, baroreceptors have been reported to reset acutely in as little as 20 min (63). Before generating an ISP-CSN activity curve, I maintained ISP below the CBr threshold for at least 20 min. It is possible that resetting occurred during this interval and that this may in part account for the low "setpoint" of guinea-pig CBr.

Effects of ASA and TZ

Table 6-1 summarizes the threshold and saturation pressures, the maximum activity and the slopes of the linear portion of the ISP-CSN activity curve in the presence of ASA (10^{-5}- 10^{-3} M) and TZ (10^{-5} -10^{-3} M). The effects of IND on CSN activity were not studied due to the poor solubility of that drug.

ASA had no effect on either the CCr discharge present below the CBr threshold or on the curve relating CBr activity to ISP. This lack of effect on either CCr or CBr challenges my hypothesis that NSAID act directly on CBr to increase their activity (Chapter V). However, there is some question as to whether ASA was delivered into the sinus in sufficient concentrations to affect CBr discharge. Krebs solution uses bicarbonate-carbon dioxide buffer. Bicarbonate facilitates ASA decomposition (210) and may have accelerated the breakdown of ASA into acetate and salicylate, contributing to the high solubility of ASA in my experiments. Sodium salicylate (i.v.) had no effect on CSN activity (Chapter V).

Tartrazine had little effect on the low-level CCr discharge below the CBr threshold, but it increased CSN activity at all ISP between threshold and saturation pressures when compared to control curves. The ISP-CSN activity curve generated in the presence of TZ (10^{-3} M) is shown in figure 6-1. Threshold pressure, maximal CSN activity, and slope were increased by TZ at all three concentrations examined, and these effects achieved statistical significance at 10^{-3} M. Saturation pressure was unchanged at all TZ concentrations.

The concentration of TZ required to increase CBr activity is greater than that for drugs such as NA (10^{-9} M) (253) and the calcium channel blocker nifedipine (10^{-5} M) (111), which have been reported to increase CBr activity by a direct action. The mechanism of action of the TZ-induced increases in CBr activity is unknown. It has been suggested that changes in smooth muscle tone may result in an increase in CBr activity 35, 126); however, there is very little smooth muscle in guinea-pig sinus (228). Although TZ increased MABP, its effect was small, unrelated to dose and not indicative of a potent vasoconstrictor action (Chapter V). In addition, other studies have shown that aortic arch baroreceptors (177) and CBr (253) are unloaded by NA-induced smooth muscle contraction. Baroreceptors respond to wall strain (53) or perhaps wall tension (63). Contraction of the smooth muscle in a vessel reduces its diameter and decreases the strain and tension on the vessel wall. In dogs, it has been shown that vasoconstrictors applied locally to the sinus decrease the diameter of the sinus (34).

One cannot define clearly a site of action using whole nerve recordings because it is impossible to extrapolate from whole nerve to single receptor units. An increase in whole nerve activity may imply two things: 1) recruitment of previously silent fibers; and 2) increase in the activity of individual receptors. There is no evidence for silent baroreceptors. Noradrenaline (177), VP (220), and nifedipine (111) have been shown to increase baroreceptor activity by a direct action. It is possible that TZ may also act by a direct action. However, in the absence of any single-fiber-preparation studies of the effects of TZ on CBr, such suggestions remain speculative.

It is unfortunate that IND was not examined in this study. Attempts to dissolve the acid or its trihydrate were ineffective and IND precipitated in Krebs solution. ASA appeared to have no effect on CSN afferents in isolated sinuses, but I am not convinced that the sinus was perfused with ASA and not with acetate and salicylate. Thus, the effects of cyclooxygenase inhibition and the possible role of PG on CSN activity remain unexplored. Prostaglandins are known to activate vagal cardiac C-fibers (119), RAR (218), and effect a negative feedback control on cholinergic activity in the airways (130, 183). Thus, PG could very well have a direct effect on CSN activity. Clearly, the effects of other cyclooxygenase inhibitors or perhaps of arachidonic acid infusion into the sinus to stimulate PG formation (119), should have been studied. However, the low success ratio and the difficulty of the preparation forced the termination of this study.

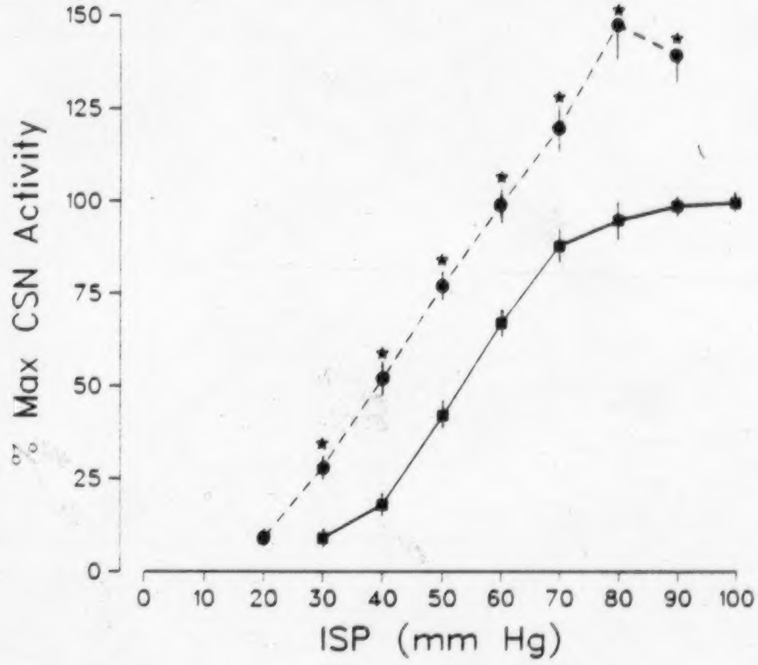

Figure 6-1: Relationship between ISP and CSN activity in the presence of Krebs solution only (solid line) and TZ at 10^{-3} M (dashed line). Each point represents the mean of four animals.

★ Significant $p \leq 0.05$

Table 6-1. Effects of ASA and tartrazine on the slope of the CSN activity - ISP curve and the baroreceptor threshold point.

DRUG	CONCENTRATION Moles/l	N	SLOPE % Activity/ mm Hg	THRESHOLD mm Hg	SATURATION mm Hg	MAXIMUM ACTIVITY % Activity
Kreb's		10	2.01 ± 0.09	31 ± 5	80 ± 4	100
ASA	10^{-5}	4	1.89 ± 0.28	28 ± 3	85 ± 5	100
	10^{-4}	4	2.10 ± 0.40	30 ± 3	80 ± 4	110 ± 6
	10^{-3}	4	2.08 ± 0.31	26 ± 5	80 ± 2	98 ± 10
Tartrazine	10^{-5}	5	2.53 ± 0.22	25 ± 5	75 ± 3	111 ± 6
	10^{-4}	4	2.86 ± 0.39	25 ± 5	75 ± 3	131 ± 10
	10^{-3}	4	3.11 ± 0.17*	18 ± 2*	70 ± 3	141 ± 12*

* Statistically significant ($p < 0.05$)

CHAPTER VII

The baroreflex and chemoreflex in guinea pigs

Introduction

In guinea pigs, unilateral orthodromic electrical stimulation of CSN is claimed to induce reflex bronchoconstriction (44). Also, CSN afferents are claimed to mediate reflex bronchoconstriction induced by TZ and NSAID (202). The findings described previously (Chapters V, VI) indicate that NSAID and TZ increase CSN activity. The purpose of this study was to examine the effects of CSN stimulation on bronchial smooth muscle tone and to evaluate the respective roles of CCr and CBr on the reflex bronchoconstriction induced by electrical stimulation of CSN.

Results

i) Bilateral occlusion of the common carotid arteries

The CCA were bilaterally occluded to examine the effects of unloading CBr stimulus on vaso- and bronchomotor tone. The CCA were occluded for 1 min with 2 small vascular clamps placed 2 cm caudal to the sinus. In spontaneously-breathing guinea pigs anesthetized with urethane or pentobarbital, and in artificially-respired guinea pigs under α-chloralose anesthesia, bilateral occlusion of the CCA induced a fall in MABP, but had no effect on HR, V_t, R_T, E_T, R_L, or E_L. The largest decreases in MABP were recorded in animals given α-chloralose, and the smallest in those given pentobarbital (Fig 7-1). Intrasinus pressure fell to 50-60 % of baseline MABP when the sinuses were occluded (Fig 7-2). Falls in MABP in response to bilateral occlusion of the CCA were unaffected by bilateral section of the ADN or the CSN in all three groups, but were abolished by bilateral vagotomy after sectioning of the ADN in urethane- and α-chloralose-anesthetized animals (Fig 7-2). In pentobarbital-anesthetized animals, bilateral vagotomy induced apnea and death.

ii) Bilateral orthodromic electrical stimulation of CSN

Both CSN were electrically stimulated to reexamine the effects of increasing CBr and CCr activity on vaso- and bronchomotor tone. Initially, CSN were stimulated at 2 and 4 V using a pulse width of 0.2 ms. Increases in R_T induced by CSN stimulation were maximal at these voltages and pulse width when a suction electrode was used (44). Stimulating CSN at these voltages should excite only myelinated afferents. The threshold for myelinated afferents in guinea pig ADN was 2 V, whereas that for the unmyelinated was 8 V when bipolar platinum electrodes were used (H.S. Sun, personal communication).

In my experiments in artificially-ventilated guinea pigs anesthetized with urethane, electrical stimulation of the CSN at either 2 or 4 V and a pulse width of 0.2 ms decreased MABP and HR, but had little effect on R_T and E_T. The decreases in MABP were greater at 4 V and the CSN were stimulated at 4 V in all subsequent studies. The decreases in MABP at 4 V were dependent upon pulse width: Threshold was about 0.05 ms and the MABP response was maximal between 0.2 and 0.4 ms. Falls in HR were small ($\leq$ 15 beats/min) and did not change when voltage or with pulse width were varied over the aforementioned ranges (Fig 7-3).

iii) Stimulation of CCr and CBr in freely-respiring pentobarbital-anesthetized guinea pigs

The effects of selectively stimulating CCr and CBr on the airways and cardiovascular system were examined in free-respiring, pentobarbital-anesthetized guinea pigs. Carotid chemoreceptors were stimulated selectively by injecting NaCN (50 - 200 μg/kg, 0.01 ml) retrogradely into the sinuses. Injecting NaCN increased V_t and f, but neither effect was dose-related (Fig. 7-4). No changes in R_L, E_L, MABP, or HR were seen in response to NaCN.

Carotid baroreceptors were stimulated in pentobarbital-anesthetized animals using bilaterally isolated sinuses perfused in tandem with the animals' own blood (ISP=50 mm Hg, flow rate 2 ml/min). Both ADN were sectioned to exclude the influence of aortic baroreceptors. Isolating the sinuses and sectioning the ADN increased MABP (from 67 ± 3 to 82 ± 5 mm Hg n=5). Intrasinus pressure was raised from 50 to 100 mm Hg in a single step and maintained for 1 min to stimulate CBr. The increase in ISP induced a fall in MABP, but had no effect on HR, R_L, E_L, V_t, or f (Fig. 7-5). Mean arterial blood pressure returned to baseline when ISP was returned to 50 mm Hg in a single step. Heart rate, R_L, E_L, V_t, and f were not altered by decreasing ISP.

Intrasinus pressure was increased in the presence of atropine (0.5 mg/kg) and mepyramine (0.2 mg/kg) to determine if the reflex vasodilation induced by CBr stimulation was cholinergic or histaminergic. Atropine and mepyramine following atropine, had no effect on baseline MABP or on the fall in MABP in response to CBr stimulation (Fig. 7-6).

iv) Stimulation of CCr and CBr in freely-respiring urethane-anesthetized guinea pigs

The effects of CCr and CBr stimulation were compared in urethane-anaesthetized guinea pigs to determine the influence of changing the anesthetic. As before, CCr were stimulated selectively by retrograde injection of NaCN (50 - 200 μg/kg) into both sinuses. NaCN increased

V_t, f, and MABP without altering HR. NaCN also induced small (≤ 40%) increases in R_L, but did not affect E_L. The V_t, f, MABP, and R_L responses to NaCN were not dose-dependent (Fig 7-7). The effects of NaCN on V_t and f in urethane-anesthetized guinea pigs were greater than in animals anesthetized with pentobarbital.

In one series of experiments, in an attempt to 'kill' the carotid body by asphyxiation, a talc suspension (300 mg/ml) was infused into the sinus to induce thrombosis of the small vessels feeding the body. Local infusion of talc suspension into the sinuses had little effect on the chemoreflex induced by NaCN in urethane-anesthetized guinea pigs. Increases in V_t and f induced by NaCN (50 μg/kg) before and after infusion of talc did not differ (Fig 7-7).

Subsequently, in a second series of experiments, carotid baroreceptors were stimulated using isolated sinuses perfused with Krebs solution (ISP=50 mm Hg and flow rate 2 ml/min). Isolating the sinuses and sectioning the ADN bilaterally raised MABP (48 ± 4 to 60 ± 3 mm Hg). Intrasinus pressure was raised from 50 to 100 mm Hg in a single step and maintained for 1 min to stimulate CBr. Raising ISP resulted in a fall in MABP and V_t. The fall in MABP began to recover while the ISP was still elevated in 2 of 5 animals. The fall in MABP in urethane-anesthetized animals was smaller than in pentobarbital-anesthetized animals. The decrease in V_t was short-lasting (5-8 breaths) in all 5 animals tested, and adapted fully in 3 of 5 animals. Heart rate decreased slightly (10 - 15 beats/min) in 3 animals, but no consistent changes in R_L, E_L, or f were noted in any of the animals (Fig. 7-8).

Decreasing ISP from 100 to 50 mm Hg in a single step increased R_L

and V_t in all 5 animals. The increase in V_t was short lasting (5-8 breaths) in all 5 animals. The R_L response lasted for about 30 s. Mean arterial blood pressure returned to baseline. Heart rate, E_L, and f were not affected by decreasing ISP (Fig 7-8).

Bilateral vagotomy reduced f (62 ± 2 to 44 ± 1 breaths/min) and increased V_t (2.3 ± 0.2 to 3.1 ± 0.2 ml) in 3 of 5 animals. The other 2 animals developed apnea and died. The effects of bilateral vagotomy on the responses to increasing and decreasing ISP varied among the 3 animals. Increasing ISP decreased MABP and V_t, but had no effect on HR, R_L, E_L, or f. The decrease in MABP was equal to that before vagotomy in 2 animals, but was lower in the third. Following vagotomy, the fall in V_t was greater in one animal, equivalent in another, and smaller in the third.

Decreasing ISP from 100 to 50 mm Hg after vagotomy increased R_L and V_t, but had little effect on HR, E_L, f. The increases in R_L were not significantly altered by bilateral vagotomy, but the increase in V_t was greater in 2 of the 3 animals. The MABP response was unaffected by bilateral vagotomy.

Discussion

These experiments were performed to determine the mechanism of action of the reflex bronchoconstriction induced by TZ and NSAID (i.v.). Tartrazine and NSAID (i.v.) were previously shown to increase CBR activity. In order to determine the effects of CBr stimulation on bronchomotor tone, a decrease and an increase in CBr activity were mimicked after eliminating the contribution of aortic baroreceptors.

In guinea pigs - as reported by others (42) - bilateral occlusion of the CCA induces a paradoxical reflex fall in MABP without altering HR. In the present study, this decrease in MABP was shown to be present in urethane-, pentobarbital-, and α-chloralose-anesthetized guinea pigs. The largest decrease in MABP was noted in the α-chloralose, and smallest in the pentobarbital group. There was no effect on HR, ventilation, or bronchomotor tone in any of the animals. This finding was surprising in view of the size of the fall in MABP (10-25 mm Hg; 15-40 % of resting MABP).

The paradoxical fall in MABP in response to bilateral occlusion was not due to local effects on either CCr or CBr fibers, as it was unaffected by bilateral CSN section. Bilateral occlusion of the CCA did reduce ISP to 50-60 % of the resting MABP. Intrasinus pressure following occlusion is determined by; 1) the degree of anastomoses between the carotid, vertebral, and cervical arteries; 2) the magnitude of the pressor response to occlusion; 3) the magnitude of the pulse pressure reduction in the sinus (214). Given that a fall, and not an increase, in MABP resulted from occlusion, and that the pulse pressure was near zero (Fig. 7-2), the pressure in the sinus must reflect a high degree of anastomoses. This rapid equilibration may account for the absence of CCr activity - evidenced by a lack of an increase in V_t and f - during occlusion. It may be suggested that, due to the partial maintenance of ISP, the loss of CBr activity may be small and undetected. This is unlikely. In other species, ISP can recover to 50-80 % of MABP (145). In these animals, an increase in MABP in response to occlusion is due to the absence of pulsatile flow and the fall in ISP (214).

The fall in MABP was unaffected by bilateral section of the ADN, indicating that it does not reflect an increase in aortic arch baroreceptor activity in response to an increased load on the aorta. Bilateral vagotomy abolished the response, suggesting that the fall in MABP is mediated by either vagal efferents or afferents.

The lack of an accompanying fall in HR mitigates against vagal efferent involvement and suggests that the fall in MABP is due solely to reflex vasodilation. Furthermore, muscarinic antagonists failed to block the fall in MABP (42). The reflex is clearly sympathetic as the responses were abolished by bethanidine and 6-hydroxydopamine (42). This vasodilation may be mediated either by increased circulating adrenaline from the adrenal medulla acting on β_2-adrenoceptors in some vascular beds - a "positive" sympathetic effect - or from a loss of α_1-adrenoceptor vascular tone - a "negative" sympathetic effect (226). If the decrease in MABP was due to increased circulating adrenaline, then bronchodilation - evidenced by a decreases in R_L and E_L - should be observed. Such decreases were never noted in response to bilateral carotid occlusion. This suggests that the fall in MABP is mediated via loss of α_1-adrenoceptor tone. One may question why withdrawal of sympathetic tone would not affect HR. It should be noted that HR did not change appreciably in response to CSN and selective CBr stimulation. Furthermore, ADN stimulation decreased HR in some, but not all animals. The absence of an effect on HR in response to a withdrawal of sympathetic tone suggests that the sympathetic system has little role in the regulation of HR in some animals.

The lack of a vagal efferent response suggests that vagal afferents may mediate this response. The question is how? The vagus does contain cardiopulmonary afferents. Stimulation of these afferents can modulate arterial baroreflexes and can induce a fall in blood pressure (162). They may be stimulated by the increased load resulting from occlusion of the CCA. However, given the alternate pathways for blood flow and rapidity with which the system in the guinea pig responds to occlusion, this load should be a transient phenomenon. By contrast, the fall in MABP was extended over the entire period of the occlusion in all but two animals.

The fall in MABP may be centrally mediated effect, but how is still unclear. Although bilateral occlusion of the CCA does deprive the CNS of its major blood supply, the collateral circulation should compensate. This is suggested indirectly by the stability of the animals in the bilaterally perfused sinus experiments, where the branches of the CCA above the sinus were cannulated or tied. If there was a mild drop leading to transient hypoxia in the CNS, one should expect an increase in MABP and not a decrease (103). To determine the nature of the CNS response to occlusion, the carotids should be bilaterally occluded above the bifurcation.

Orthodromic electrical stimulation of the CSN was undertaken to determine the effects of increasing CBr activity. Bilateral CSN stimulation produced a classic reflex fall in MABP accompanied by small decreases in HR. This suggests that the fall in MABP is mediated by sympathetic efferents. In guinea pigs, when ADN are stimulated electrically, MABP and HR fall due to withdrawal of sympathetic tone (H.S. Sun, personal communication). In rats, the fall in MABP in response to CSN stimulation results from CBr-induced reflex vasodilation mediated via a histaminergic vasodilator mechanism; the fall in HR is due to increased vagal tone (153). However, in guinea pigs, the fall in MABP induced by increased CBr activity was unaffected by atropine and/or mepyramine. Thus, the vasodilation due to CSN stimulation - like that induced by ADN stimulation - was mediated via a withdrawal of sympathetic tone and has neither a histaminergic nor a cholinergic component.

Unilateral orthodromic electrical stimulation of a CSN mimics the effects of TZ and NSAID and increases R_T (44). In the present study, bilateral orthodromic stimulation of CSN failed to alter airway smooth muscle tone even when the ADN were sectioned. The increases in R_T reported previously were small (44). Bilateral stimulation should increase the size of any responses because it avoids the effects of "pendelluft". An increase in CBr activity could decrease the release of catecholamines from the adrenal medulla. It may be argued that the withdrawal of circulating catecholamines should induce bronchoconstriction. This argument assumes that in guinea pigs airway caliber is under tonic sympathetic control. However, in normal guinea pigs the sympathetic system serves a "defensive" role mediating the recovery from challenges to the airways (277). This may explain the discrepancy between the results reported here and in the previous study. Shortly after the original experiments were completed, the entire guinea pig colony developed a serious infection and was destroyed. In normal guinea pigs, the non-specific β-blocker propranolol has no effect on airway smooth muscle, although it does potentiate the actions of agonists (Chapter IX). By contrast, propranolol increased R_T in ovalbumin-sensitized guinea pigs - an experimental model of asthma (Chapter IX). Thus, the effects of CBr-induced decrease in sympathetic tone on the airways may be detectable only in sensitized animals. The lungs of the animals used in the present study were free of infection on histological examination (W.C. Hulbert, personal communication).

In order to distinguish between the effects of CCr and CBr stimulation, I tried to stimulate CCr selectively by injecting NaCN retrogradely into the sinus. Stimulation of CCr in freely-respiring pentobarbital-anesthetized animals induced characteristic increases in V_t and f, but had little effect on airway and vascular smooth muscle. By contrast, CCr stimulation in urethane-anesthetized animals produced an increase in R_L and MABP as well as the characteristic increase in ventilation. Moreover, the increases in V_t and f in the urethane-anesthetized group were greater than those in animals treated with pentobarbital, which suggests that pentobarbital attenuates the chemoreflex in guinea pigs. Although some anesthetics are known to interfere with the ventilatory responses to peripheral chemoreceptor stimulation, pentobarbital is reported to have little effect (89). The attenuation of the chemoreflex reported may be a manifestation of the respiratory depressant effects of pentobarbital in guinea pigs.

It is noteworthy that diazoxide and 5-HT have also been reported to increase bronchomotor tone via CSN afferents (201). Diazoxide is a potent vasodilator and may increase CCr discharge indirectly by its severe effects on MABP. 5-HT increases CCr discharge by non-specific actions on sensory neurons (85, 144). The increase in R_T reported in response to diazoxide and 5-HT may be due to CCr activity.

The increases in MABP suggest that CCr stimulation may increase sympathetic tone. However, the increases in MABP were small and HR was not increased. This suggests that CCr have only small reflex effects on sympathetic tone. An increase in sympathetic tone also seems unlikely to account for the increase in R_L. Given the dominance of β-adrenoceptors in the airways, an increase in circulating catecholamines should induce bronchodilation (277).

Carotid baroreceptors were stimulated using an isolated, perfused carotid sinus. The advantage of this preparation is that it offers independent control of ISP and separates the input from the reflex effects on the vasculature. However, in guinea pigs it was impossible to physically separate the carotid sinus from the carotid body in order to exclude the effects of CCr on the CBr responses. One way to attack this problem is to control CCr activity by autoperfusing the sinuses in animals breathing O_2-enriched air or 100 % O_2 and carefully monitoring the expired gases (50). Alternatively, one could try to eliminate CCr by destroying the carotid body. I was unable to destroy the carotid body by infusing a talc suspension as described by Franz (92). However, neurophysiological studies showed that in a well-oxygenated sinus, CCr activity should only be present below CBr threshold (Chapter VI). Since the resting ISP was always maintained above CBr threshold (30 mm Hg) and the sinus was not left unperfused for more than 3 min at a time, I assumed that CCr activity was stable when the sinus was autoperfused or perfused with oxygenated Krebs solution.

In both urethane- and pentobarbital-anesthetized animals, increasing ISP induced a characteristic fall in MABP without altering HR.

Mean arterial blood pressure returned to baseline when ISP was decreased. Loading and unloading CBr in this fashion induced short-lasting changes in the opposite direction in V_t in urethane-, but not in pentobarbital-anesthetized guinea pigs. Rate of respiration was unaffected in both groups of animals. An increase in R_L was noted when ISP was decreased in urethane-anesthetized guinea pigs only. The differences between the ventilatory and airway-smooth-muscle responses to changes in ISP in pentobarbital and urethane-anesthetized groups were not unexpected. Pentobarbital anesthesia has been reported to attenuate the reflex effects of baroreceptor stimulation on ventilation in dogs (54) and on smooth muscle tone in cats (68).

The literature on the effects of CBr stimulation on airway-smooth-muscle tone is inconsistent. Daly and Schweitzer (68) showed that stimulation of CBr produces bronchoconstriction in dogs and cats; others have reported a small bronchodilation in response to increases in ISP in dogs (182). In both studies, the reflex effects of CBr on bronchomotor tone were stated to be mediated by vago-sympathetic efferents. In dogs, a recent study has shown that increasing or decreasing ISP about the normal setpoint of 100 mm Hg, induced inverse changes in tracheal smooth muscle tone via a vagal cholinergic pathway (222). Changes in tracheal tension showed a dynamic phase, which adapted to a new steady-state and persisted until ISP was restored to setpoint. In guinea pigs, increasing ISP from 50 to 100 mm Hg had little effect on bronchomotor tone. Decreasing ISP back to 50 mm Hg induced only a transient increase in bronchomotor tone. Furthermore, bilateral carotid occlusion had no effect on airway patency. These findings indicate that in guinea pigs, CBr have no tonic influences on lower airway patency; however, the effects of CBr stimulation on tracheal smooth muscle tone remain to be investigated in this species.

There is some evidence to suggest that the transient changes in R_L and V_t seen in response to changes in ISP are not due to CBr, but to CCr. When ISP was suddenly raised or lowered, chemoreceptor activity changed in the opposite direction within the first 5 - 10 s (46). Thus, decreasing ISP should increase CCr activity transiently. This transient change in CCr activity explains in part the short-lasting changes in V_t in response to increasing and decreasing ISP and the increase in R_L seen in response to a drop in ISP in guinea pigs. The R_L responses to a fall in ISP and to CCr stimulation were of the same size and present only in urethane-anesthetized animals. Moreover, the V_t response seen in guinea pigs adapted rapidly and the MABP response adapted slowly. In dogs, where the sinus and the body can be surgically separated, increasing ISP results in a decrease in f and $\dot{V}_e$, and an increase in V_t, while decreasing ISP has the opposite effects (54, 138). The $\dot{V}e$ and MABP response, like the changes tracheal tension in response to CBr loading and unloading, show a dynamic phase, which adapts to a new steady-state. By contrast, in cats, in which the sinus and body are contiguous, increasing ISP results in a transient decrease in f and V_t which returns to baseline and a stable decrease in MABP (50). It has been suggested that the differences in the ventilation and cardiovascular responses to changes in ISP reflect differences in the central nervous system processing of the same input. I propose that in cats and in guinea pigs the transient ventilatory and the slowly-adapting MABP responses induced by changing ISP may reflect CCr and CBr stimulation, respectively.

If CCr, and not CBr, increase bronchomotor tone, then how do TZ and NSAID work? I propose that TZ and NSAID do act on CBr afferents to increase bronchomotor tone via a withdrawal of sympathetic tone. An increase in R_T induced by this withdrawal of sympathetic tone may only be evident in sensitized animals when sympathetic tone is present in the airways. This effect is only present in sick animals. The bronchoconstriction in response to stimulation of CSN in sick animals is due to a summing of the withdrawal of sympathetic tone and the bronchoconstrictor actions of CCr.

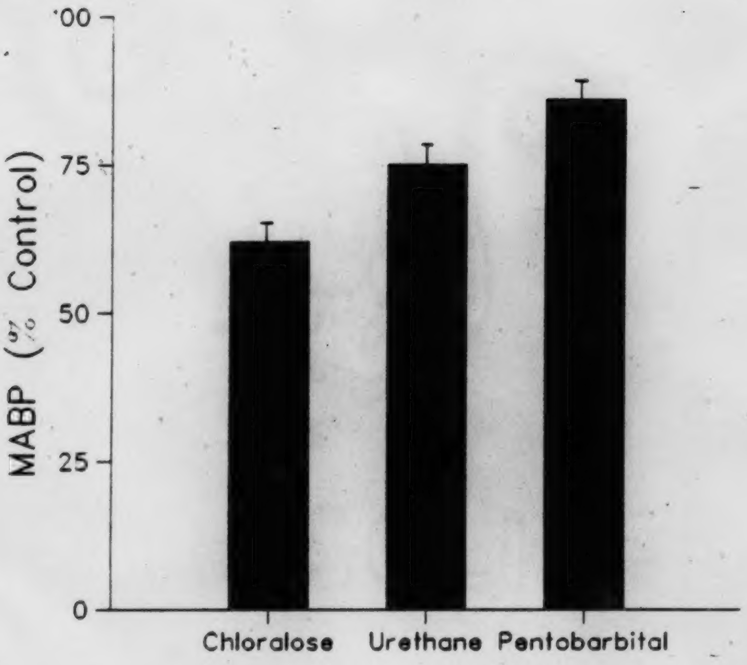

Figure 7-1: Effects of different anesthetics on the maximal decreases in MABP induced by bilateral occlusion of the common carotid arteries in guinea pigs. Baseline MABP in these animals were: α-chloralose - 70 ± 3 mm Hg; urethane - 48 ± 4 mm Hg; pentobarbital - 64 ± 3 mm Hg. Bars represent mean ± s.e.m., n=4.

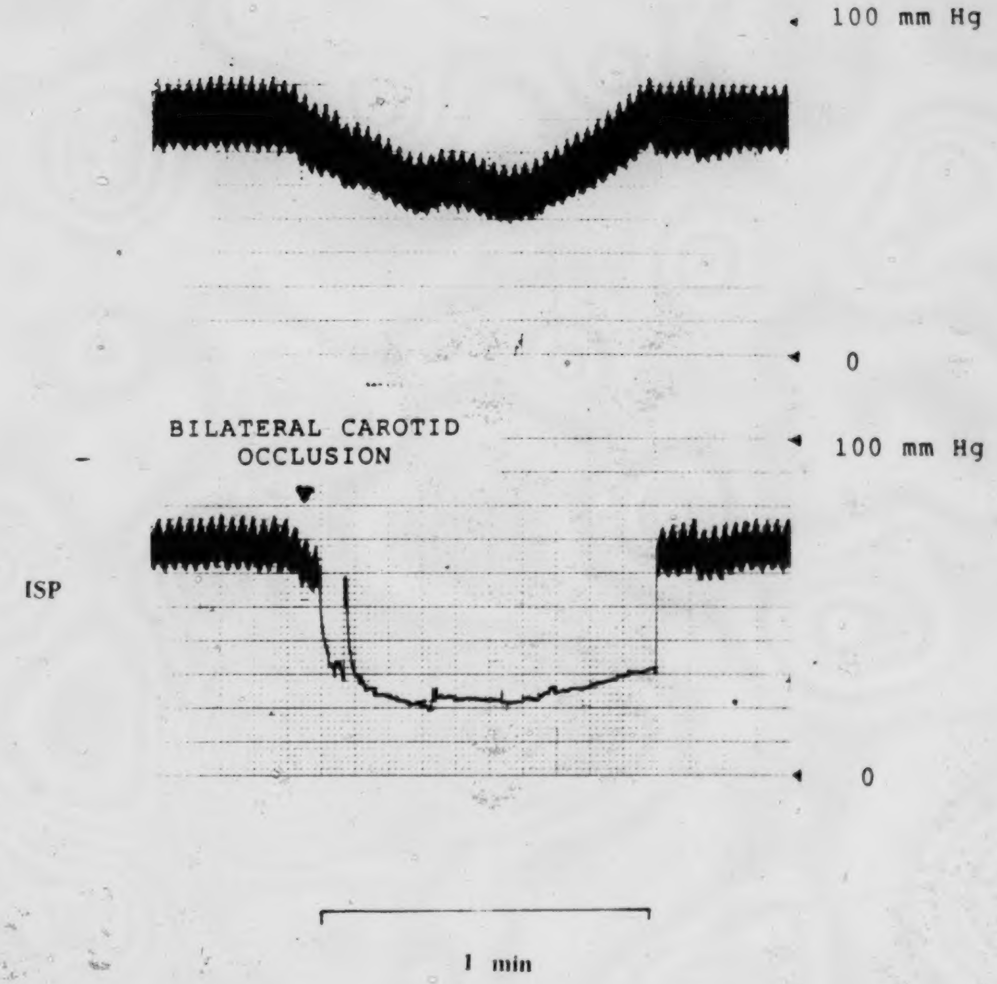

Figure 7-2: Tracings of ISP and arterial blood pressure responses during bilateral carotid occlusion in guinea pigs anesthetized with α-chloralose.

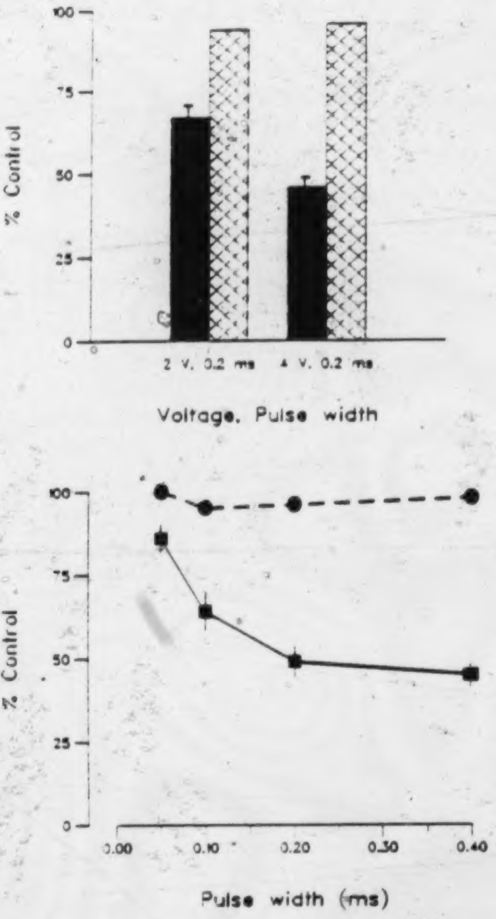

Figure 7-3: a) Mean arterial blood pressure (MABP) (solid bar) and HR (hatched bar) responses to bilateral electrical CSN stimulation (2 or 4 V; pulse width, 0.2 ms). Bars represent mean ± s.e.m. b) The effects of pulse width on MABP (■, ——) and HR (●, ---) responses to bilateral electrical CSN stimulation at 4V. Control values of MABP and HR were: 44 ± 2 mm Hg and 268 ± 8 beats/min. Each point represents the mean of 4 animals.

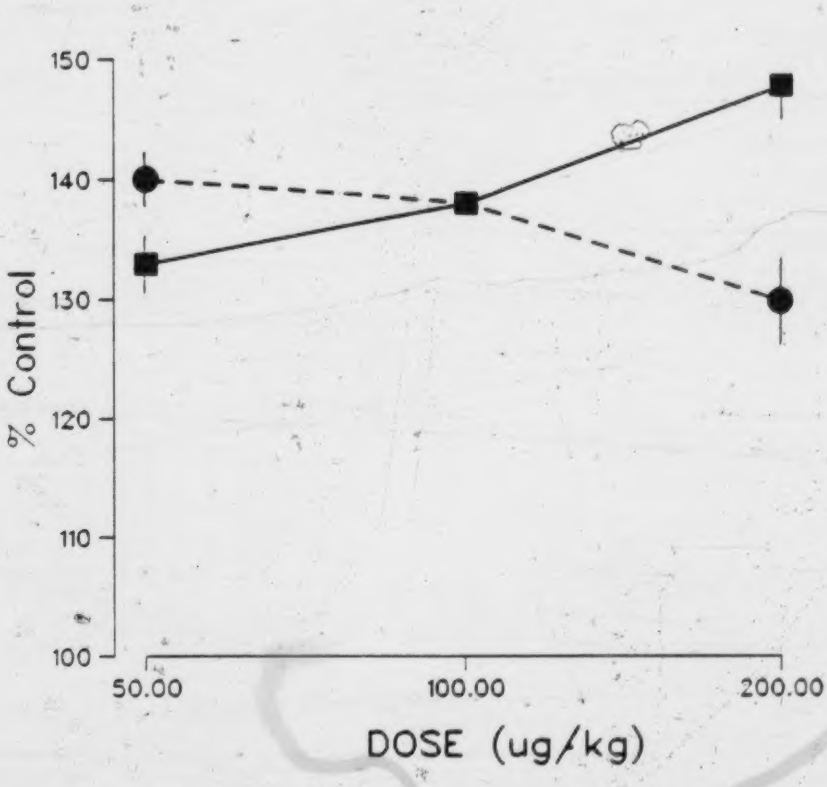

Figure 7-4: Effects of NaCN (50-200μg/kg) on V_t (■, ——) and f (●, ---) in pentobarbital-anesthetized animals. Control values for V_t and f were: 3.44 ± 0.22 ml and 36 ± 3 breaths/min. Each point represents the mean of 5 animals.

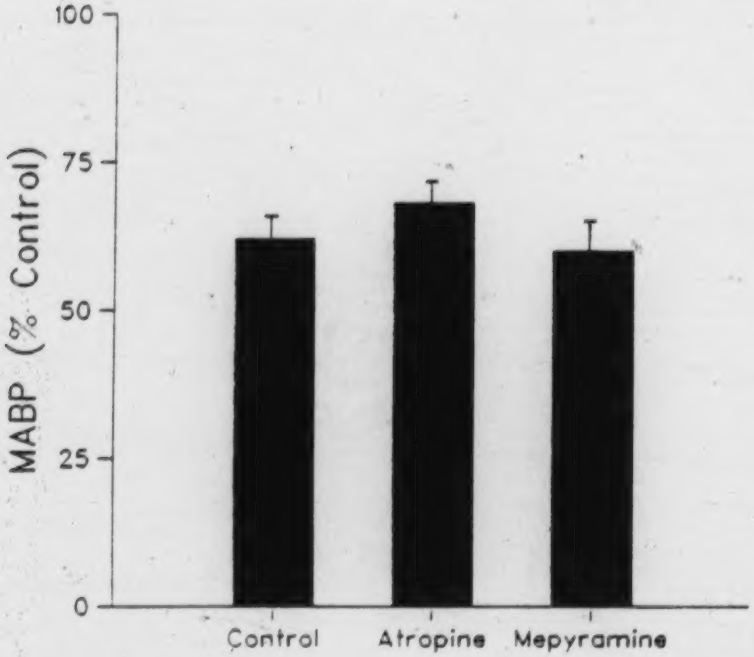

Figure 7-5: Effects of increasing ISP on MABP in the absence of drug (Control), in the presence of atropine (0.5 mg/kg), and atropine and mepyramine (0.2 mg/kg). Baseline MABP was 82 ± 5 mm Hg. Each point represents the mean of 5 animals.

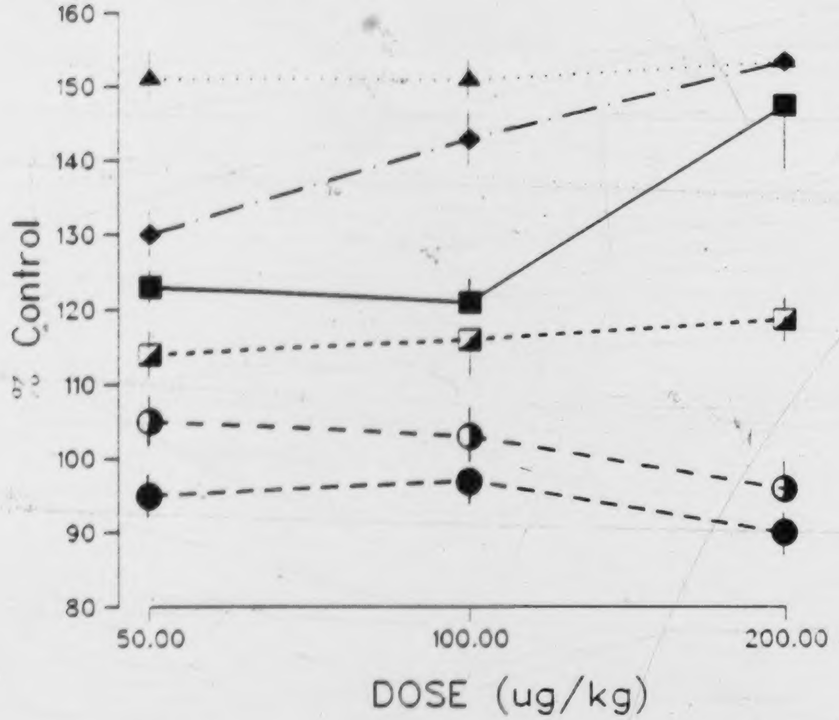

Figure 7-6: Effects of NaCN on V_t (▲), f (◆), MABP (◪), HR (◑), R_L (■), and E_L (●) in urethane-anesthetized guinea pigs. Baseline values were: V_t - 2.45 ± 0.13, f - 71 ± 6, MABP - 46 ± 3, HR - 285 ± 12.

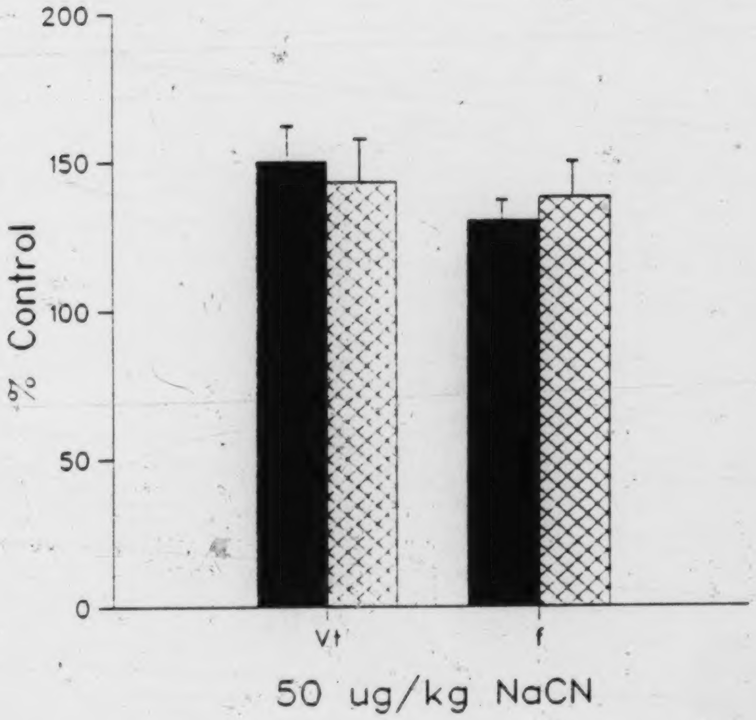

Figure 7-7: Effects of NaCN on V_t and f before (solid bars) and after (hatched bars) infusion of talc into the sinus in 5 urethane-anesthetized animals. Baseline values were V_t - 2.45 ± 0.13 and f- 71 ± 6.

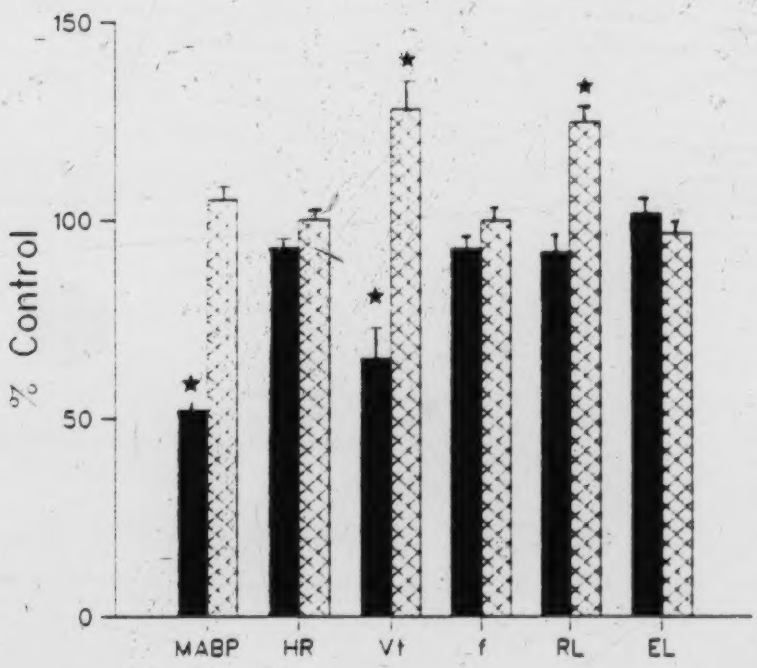

Figure 7-8: Effects of increasing (closed bars) and decreasing (hatched bars) ISP on MABP, HR, V_t, f, R_L, and E_L in 5 urethane-anesthetized animals. Baseline values were: V_t - 2.63 ± 0.14, f - 68 ± 6, MABP - 46 ± 3, HR - 297 ± 10. ★ Statistically different from baseline levels.

CHAPTER VIII

Effects of Angiotensins II and III on respiration in guinea pigs

Introduction

The effects of angiotensin II (AII) on the cardiovascular system have been reviewed (77). In addition to its powerful direct effects on vascular smooth muscle, AII facilitates transmission in sympathetic ganglia, and induces the catecholamine release from the adrenal medulla. More recently, AII has been found to act within the central nervous system to modulate peripheral cardiovascular responses (209).

This study was undertaken to examine the effects of increasing systemic arterial blood pressure on bronchomotor tone and to determine the role of arterial baroreceptors on any responses. Initially, phenylephrine was chosen as the pressor agent; however, it had little effect on bronchomotor tone (Chapter IX), thus, a decision was made to use another pressor agent. Angiotensin II was chosen arbitrarily. Subsequently, it was learned that the effects of AII on respiration have not been as well defined as its effects on the cardiovascular system. Early reports claimed that AII stimulated (213) or decreased respiration (185). More recently, studies in dogs showed that AII stimulates respiration, but this effect could only be demonstrated consistently after baroreceptor denervation (9, 203). It was suggested that the stimulatory effect of AII on ventilation was opposed by its pressor actions and that this accounted for the differing responses reported in the early literature. The increase in ventilation in dogs was independent of vagal afferents and efferents, and appeared to be a central effect of AII (9). In cats, AII excites neurones in the nucleus of the solitary tract that are involved in the control of respiration (224). Angiotensin III (AIII) is more potent than AII in the central nervous system and it is suggested that AIII, and not AII, may be the centrally active form of angiotensin (110).

Angiotensin II constricts guinea-pig airway smooth muscle (38, 132), but has no effect on dog airways (32). This suggests that AII may have a different role in guinea pigs. The purpose of this study was to determine the effects of AII on respiration and airway-smooth-muscle tone in guinea pigs and to determine to what extent baroreceptors mediated or antagonized these effects.

Results

i) Baseline Values

Average baseline values for V_t, f, R_T, R_L, E_T, E_L, MABP, and HR monitored in the guinea pigs used in this study are summarized in Table 8-1.

ii) Effects of angiotensin II in guinea pigs anesthetized with α-chloralose

The effects of AII on airway smooth muscle were first examined in artificially-ventilated guinea pigs anesthetized with α-chloralose. AII (5-20 μg/kg, i.v.) induced dose-dependent increases in R_T, E_T, and MABP. Heart rate fluctuated after AII administration, but no definite pattern was observed. Propranolol (2 mg/kg, i.v.) was given to block any bronchodilator effects of catecholamines released from the adrenal medulla by AII. Propranolol had no effect on R_T and E_T, but reduced HR and induced a transient decrease in MABP. In the presence of propranolol, AII increased R_T, E_T, and MABP at a lower dose range (0.01 - 5.00 μg/kg), and induced about a 10-fold shift of the dose-response curve to the left (Fig. 8-1). All three responses were dose-dependent. Thoracic resistance and E_T responses to AII were only seen at doses which produced maximal increases in MABP. AII had no effects on HR in the presence of propranolol.

All subsequent experiments to determine the effects of nerve sections and blockers on responses to AII were performed in the presence of propranolol. The AII response was unaffected by bilateral vagotomy, section of the ADN and CSN, or vagotomy following ADN and CSN section ($p \geq 0.20$) (Fig. 8-2). Also, hexamethonium (10 mg/kg, i.v.), a ganglionic blocker, had little effect on the AII-induced increases in R_T and E_T ($p \geq 0.80$), but it did potentiate the MABP response ($p = 0.05$) (Fig. 8-3). A specific blocker of AII, sar^1-val^5-ala^8-angiotensin II (50 μg/kg, i.v.), abolished all three responses (Fig.8-4).

The effects of AII were also studied in animals pretreated with reserpine (2.5 mg/kg, i.p.). Animals pretreated with reserpine had a lower MABP and HR, than untreated animals (Table 8-1). In these chemically sympathectomized animals in the absence of propranolol, increases in MABP, R_T, and E_T induced by AII were not statistically different ($p > 0.50$) from those induced by AII in untreated animals after β-blockade (Fig. 8-5).

Having established that AII increases R_T and E_T, I evaluated its effects on ventilation. Of 7 freely-respiring guinea pigs anesthetized with α-chloralose, 3 stopped breathing before the first dose of AII was administered and had to be placed on a respirator. In the other 4 animals, AII (0.005 - 0.500 μg/kg, i.v.) in the absence of propranolol, severely depressed respiration and 3 of the animals died before they could be placed on a respirator. The depression appeared to be independent of dose - often only one dose could be administered per animal.

iii) Effects of angiotensin II and III in urethane-anesthetized guinea pigs

Because of the respiratory depressant effects of α-chloralose in guinea pigs, I elected to reevaluate the effects of AII on ventilation in freely-respiring urethane-anesthetized guinea pigs. The effects of AIII were evaluated to determine if the ventilatory actions of AII were due to a central effect which may be mediated via AIII.

Prior to studying the effects of AII on ventilation, its actions and those of AIII were evaluated in artificially-ventilated, urethane-anesthetized guinea pigs. Angiotensin II (5 - 20 μg/kg, i.v.) and AIII (5 - 100 μg/kg, i.v.) induced dose-dependent increases in R_T, E_T, and MABP (Figure 8-6, 8-7). Propranolol (2 mg/kg, i.v.) enhanced the actions of AII and AIII on R_T, E_T, and MABP. In the absence and in the presence of propranolol, AII was about 8-10x more potent that AIII.

The effects of AII on R_T and E_T in the absence of propranolol were not affected by the anesthetic. However, AII was less effective on R_T, E_T, and MABP in the presence of propranolol in urethane-anesthetized guinea pigs ($p=0.04$). In the presence of propranolol, the MABP responses were not altered by the type of anesthetic.

In freely-respiring, urethane-anesthetized guinea pigs treated with propranolol, AII (0.01 - 5.00 μg/kg, i.v.) increased R_L, E_L, and f, and decreased V_t.(Fig. 8-8, 8-9). The R_L, E_L, f, and V_t responses were dose-dependent. The AII responses were unaffected by bilateral section of the ADN and CSN , but increases in f were abolished and the decrease in V_t were reduced by bilateral vagotomy after bilaterally sectioning the ADN and CSN (Figure 8-9) . Pulmonary resistance and E_L were not monitored after nerve section in the freely-respiring animals due to technical difficulties with the esophageal cannula. Bilateral vagotomy depressed f (97 ± 12 to 49 ± 9 beats/min) and increased V_t (from 2.2 ± 0.2 to 2.7 ± 0.3 ml)

In freely-respiring animals, AIII (5 - 100 μg/kg) had similar effects to AII (Figure 8-8, 8-10), but AII was more potent (10x) than AIII. Baroreceptor denervation had no effect on the decrease in V_t and the increase in f induced by AIII. Bilateral vagotomy after baroreceptor denervation abolished the increase in f and reduced the decrease in V_t.

Discussion

It has been previously reported that AII is a weak constrictor of guinea-pig airway smooth muscle in vivo (132) and in vitro (38), but is reported to have no effect on dog tracheal smooth muscle (32). The results of the present study demonstrate that in guinea pigs AII (i.v.) increases vascular and airway-smooth-muscle tone, but has little effect on HR. The actions of AII in the absence of propranolol were unaffected by the anesthetic, as the AII-induced increases in R_T, E_T, and MABP guinea pigs anesthetized with urethane or α-chloralose, did not differ statistically. The vascular and respiratory responses were potentiated by propranolol. A similar potentiation of the actions of AII was seen in guinea pigs treated with reserpine. This suggests that catecholamines released by the adrenal medulla in response to AII, antagonize

AII-induced hypertension and bronchoconstriction in guinea pigs, and may in part explain the small size of the airway constrictor responses reported in guinea pigs (132) and the lack of responses in dogs (32).

In urethane-anesthetized animals, airway and vascular responses to AII in the presence, but not in the absence of propranolol, were smaller than those in guinea-pigs anesthetized with α-chloralose. This discrepancy is difficult to explain. The differences may be due to the antagonistic effects of urethane on α_2-adrenoceptors (174). α_2-adrenoceptors modulate transmission through peribronchial ganglia and cholinergic terminals (106).

In the presence of propranolol, AII-induced increases in airway smooth muscle tone were apparent only when the vascular response was maximal. Neither response was unaltered by bilateral vagotomy, baroreceptor denervation, vagotomy following baroreceptor denervation, or ganglionic blockade. Both the bronchoconstrictor and hypertensive effects of AII were abolished by the specific antagonist sar^1-val^5-ala^8-angiotensin II. These findings indicate that AII's effects on the airways are independent of its action on the vasculature. They do not exclude the possibility that AII may be acting indirectly by releasing neurotransmitters from nerve terminals or varicosities. Angiotensin II is known to stimulate autonomic ganglion cells and to facilitate catecholamine release (77). However, AII may have a direct action on airway smooth muscle. If one assumes that the airways and the vasculature are exposed to the same concentration of the drug, a direct action of AII implies that it is not as potent an agonist on airway smooth muscle as on the vasculature. The difference in potency may be a reflection of different affinities of the receptors for AII. It may also be a reflection of the efficiency of the stimulus response mechanism in the tissues. Two factors which determine this efficiency are the number of receptors available and the nature of the system translating receptor stimulus into tissue response (142).

The effects of AII and AIII on R_L, E_L, MABP were similar in urethane-anesthetized animals. In other species, AIII is about one-tenth as potent as AII the vasculature (77). In guinea pigs, my findings show that AIII is less potent than AII on vascular and airway smooth muscle.

The effects of AII and AIII on ventilation were examined in spontaneously breathing guinea pigs anesthetized with urethane because of the respiratory depressant effects of α-chloralose in this species (Chapter III). All experiments were done in the presence of propranolol to exclude any effects of circulating catecholamines. Administration of AII and AIII induced rapid shallow breathing. Ventilation decreased at the lower doses and increased at the highest dose. The increases in R_L, E_L, and MABP in freely-respiring animals were statistically similar to those in artificially-ventilated guinea pigs in the presence of propranolol.

Baroreceptor denervation had little effect on respiratory responses to AII and AIII respiratory responses, but did potentiate the increase in MABP. This demonstrates that baroreceptors do not "dampen" the respiratory stimulant effect of AII and AIII, confirming my earlier finding that baroreceptors appear to play little role in control of respiration in guinea pigs. This is a noteworthy difference between this species and dogs (54, 138).

Bilateral vagotomy following baroreceptor denervation abolished the increases in f induced by AII and reduced the drug-induced decrease in V_t. This demonstrates that the effects of AII and AIII on ventilation are mediated via vagal afferents that reflexly increase f and decrease V_t. This again is a significant departure from findings in dogs, in which bilateral vagotomy following baroreceptor denervation had no effect on the stimulatory effects of AII on ventilation (9, 203).

The effects of AIII on ventilation were also abolished by bilateral vagotomy, further demonstrating that the site of action of the angiotensin-induced increase in ventilation is peripheral. It has been suggested that AIII and not AII, is the centrally-acting angiotensin metabolite (110). Because of the peripheral site of action of the angiotensin-induced increased ventilation, I was unable to determine whether AII or AIII is the centrally acting angiotensin in guinea pigs.

The vagal afferents excited by AII to increase ventilation are unknown. Likely candidates are pulmonary afferents. The rapid shallow breathing induced by AII is similar to that induced by C-fiber stimulation (194). Pulmonary C-fibers respond to increases in pulmonary capillary pressure. Angiotensin II and III are potent pressor agents and may very well increase pulmonary capillary pressure to stimulate pulmonary C-fibers to indirectly produce rapid-shallow breathing. However, the effects of AII on pulmonary vasculature were not studied and I have no evidence to substantiate or to discredit this possibility.

The increase in f and the decrease in V_t are also similar to those evoked by SAR and RAR stimulation. Both pulmonary receptors may be activated by an increase in airway smooth muscle tone. Stimulation of

RAR in the lower respiratory tract below the carina induces hyperpnea (62, 271). Increased SAR discharge can result in a decrease in Vt (62). It is noteworthy that the decrease in Vt induced by AII was reduced, but not abolished, by bilateral vagotomy. This suggests that the decrease in V_t may be in part due to the increased airway tone induced by AII. Thus, both the increase in f and the decrease in V_t result from the bronchoconstrictor effects of AII. The increases in f may be due to the indirect activation of RAR, and the decrease in V_t to a direct effect and indirect stimulation of SAR by the increased airway smooth muscle tone.

Table 8-1. Summary of mean values (± s.e.m.) of resistance (R_T, R_L), elastance (E_T, E_L), mean arterial blood pressure (MABP), and heart rate (HR) in α-chloralose- and urethane-anesthetized guinea pigs

	N	MABP mm Hg	HR beats/min	R cm H_2O/s/ml	E cm H_2O/ml	V_t ml	f breaths/min
Untreated α-chloralose artificially-ventilated	24	67 ± 2	258 ± 1	0.372 ± 0.033	1.386 ± 0.124	--	--
Reserpine α-chloralose artificially-ventilated	4	48 ± 2*	173 ± 5*	----	-----	--	--
Untreated urethane artificially-ventilated	4	48 ± 6	274 ± 8	0.299 ± 0.038	1.882 ± 0.087	--	--
Untreated urethane freely-respiring	8	47 ± 3	295 ± 12	0.267 ± 0.018	2.779 ± 0.542	2.68 ± 0.12	64 ± 6

* $p < 0.05$ Significantly less than in untreated α-chloralose-anesthetized artificially-ventilated animals

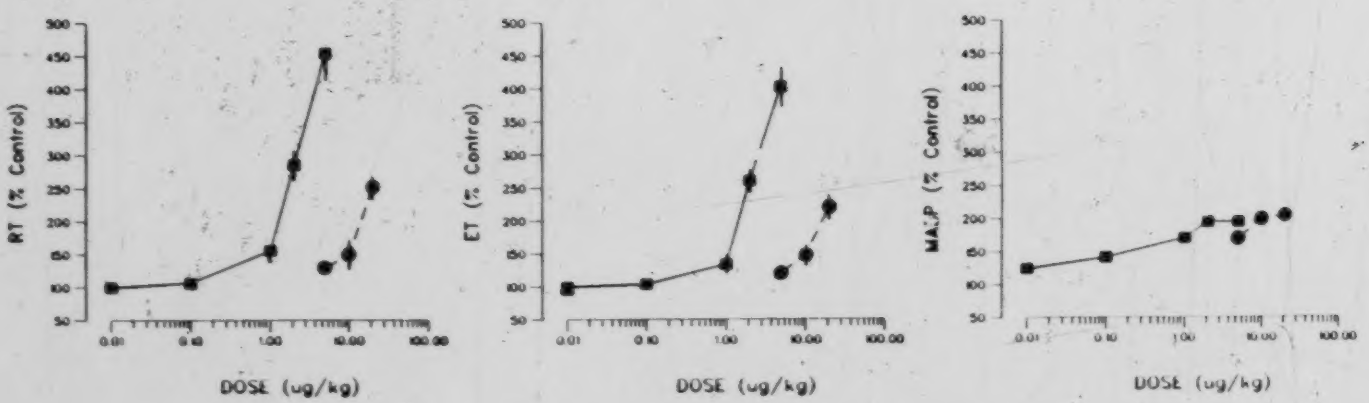

Figure 8-1: Effects of AII on R_T, E_T, and MABP before (●,---) and after (■,—) propranolol in artificially-ventilated, α-chloralose-anesthetized guinea pigs. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals.

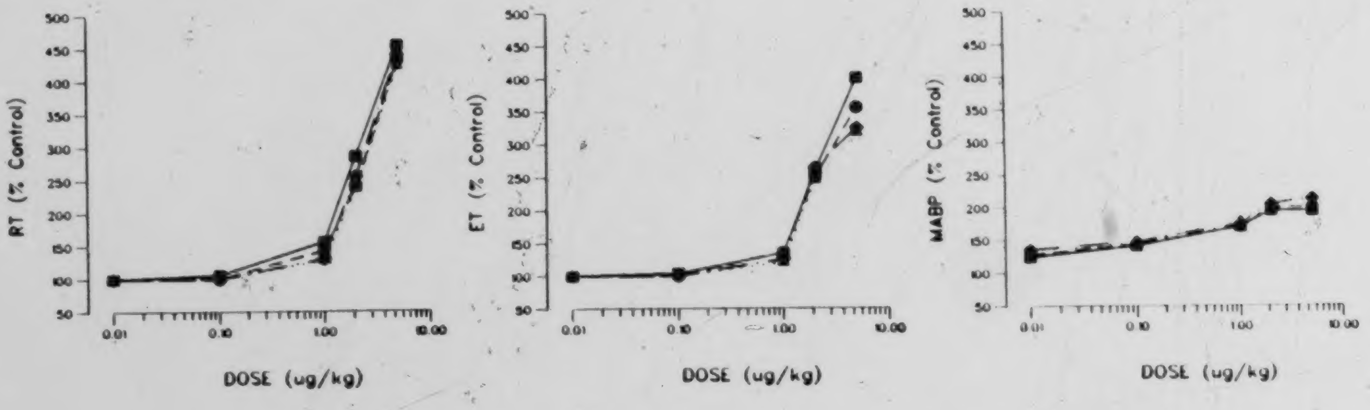

Figure 8-2: Effects of nerve sectionings on AII-induced increases in R_T, E_T, and MABP in artificially-ventilated α-chloralose-anesthetized guinea pigs. Control (■, —) bilateral vagotomy (◆, —·—), ADN and CSN section (●, ---), and bilateral vagotomy + ADN and CSN section (▲, ···). Nerve sectioning did not significantly alter the R_T, E_T or MABP to AII. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals.

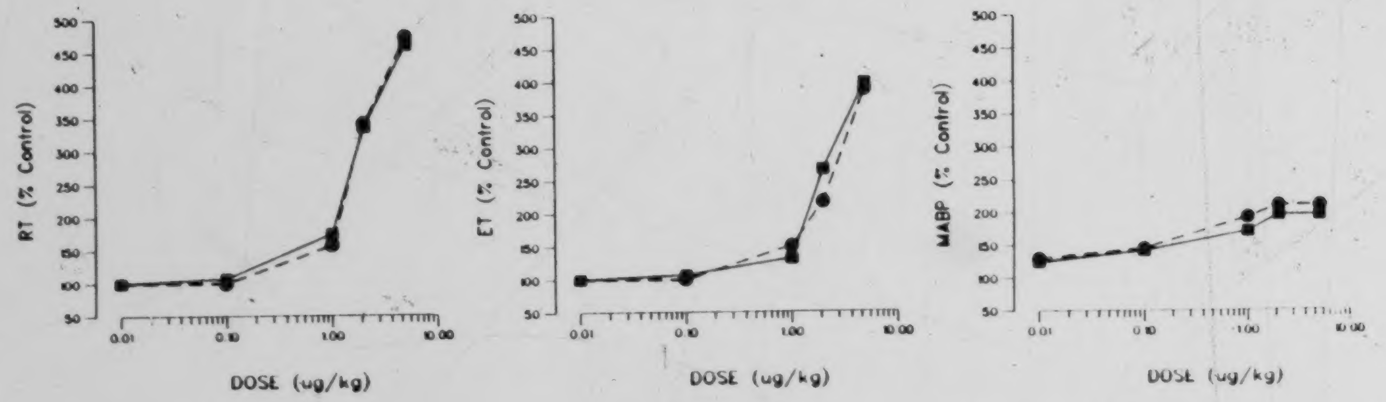

Figure 8-3: Effects of hexamethonium on AII-induced increases in R_T, E_T, and MABP in artificially-ventilated α-chloralose-anesthetized guinea pigs. Control (■, ——), Hexamethonium (●, ----). Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals.

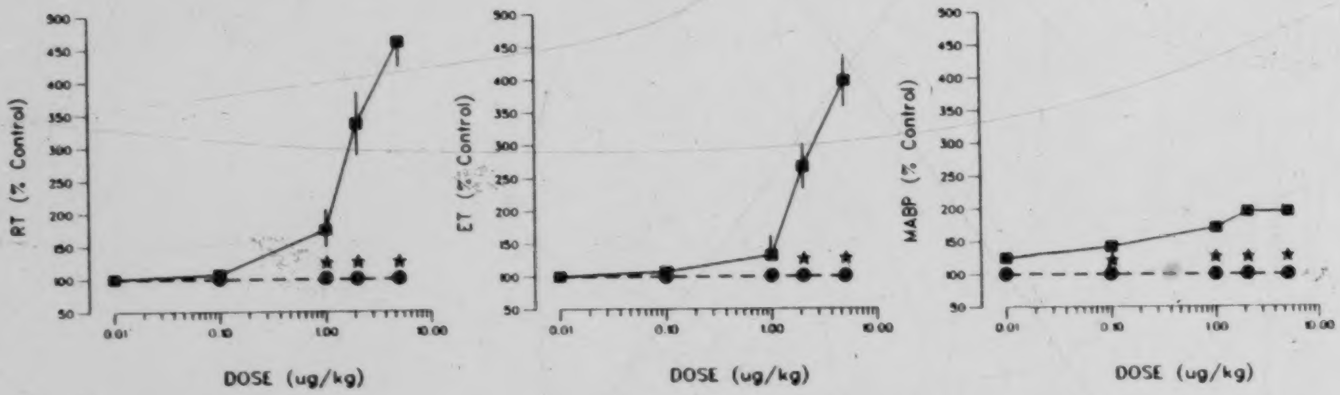

Figure 8-4: Effects of sar^1-val^5-ala^8-angiotensin II (50 μg/kg) on AII- induced increases in R_T, E_T, and MABP in artificially-ventilated α-chloralose-anesthetized animals. Control (■, —) and sar^1-val^5-ala^8-angiotensin II (●, ---). Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals. ★ Statistically significant $p<0.05$.

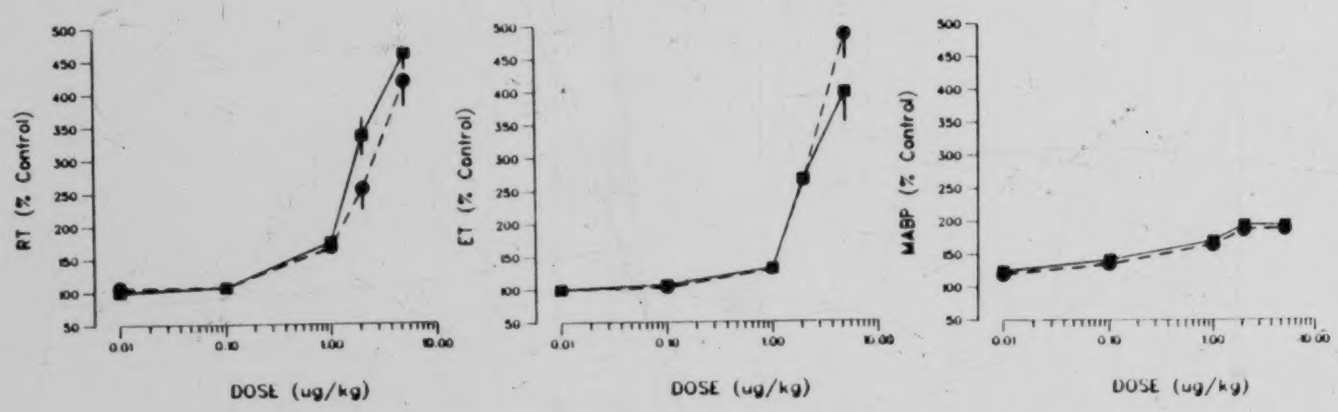

Figure 8-5: Effects of AII on R_T, E_T, and MABP in untreated animals in the presence of propranolol (■, ——) and chemically-sympathectomized animals in the absence of propranolol (●, ----). All animals were artificially-ventilated and anesthetized with α-chloralose. Increases in R_T, E_T and MABP induced by AII did not differ between these two groups of animals. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals.

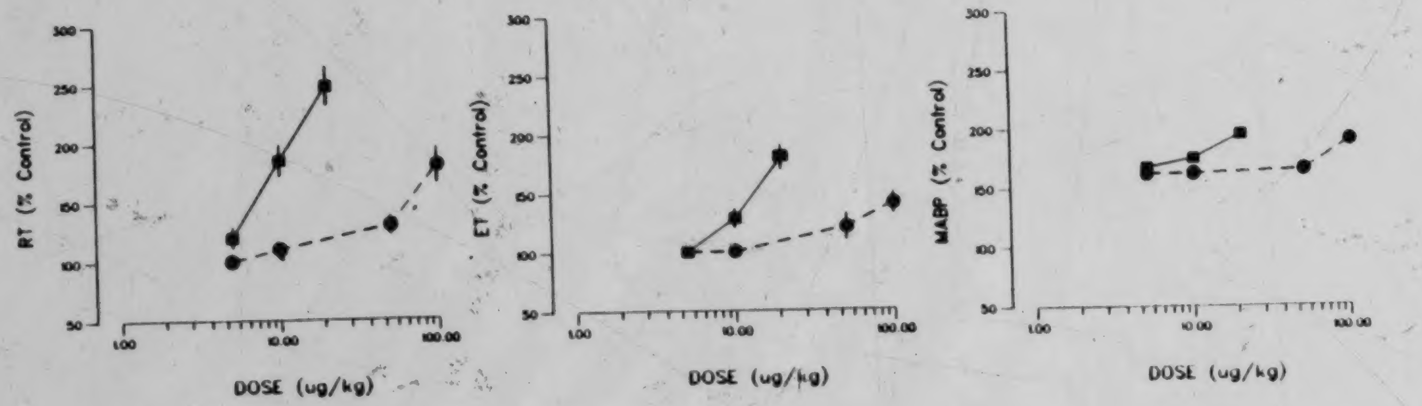

Figure 8-6: Effects of AII (■, —) and AIII (●,---) on R_T, E_T, and MABP in artificially-ventilated urethane-anesthetized animals. Baseline values are summarized in table 8.1. Each point represents the mean of 4 animals.

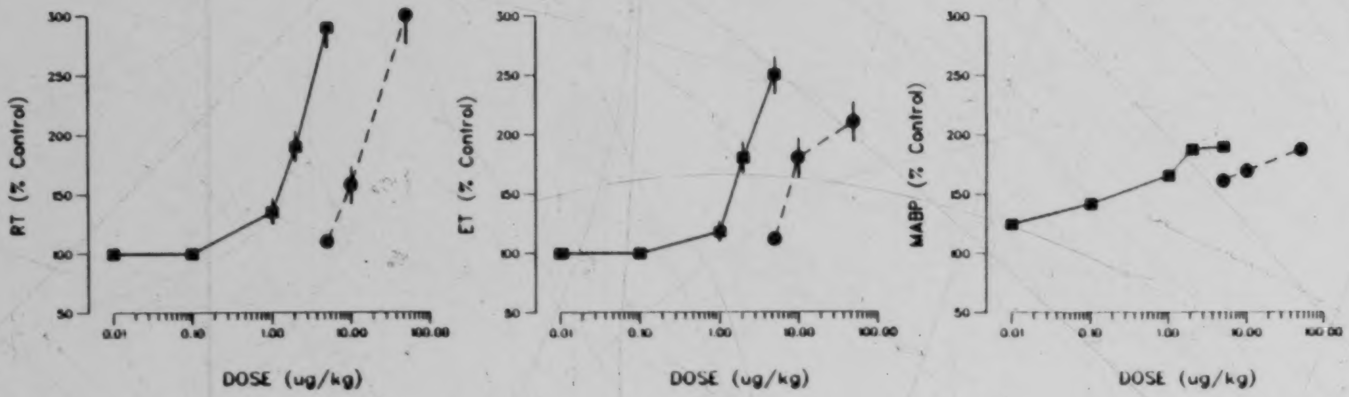

Figure 8-7: Effects of AII (■, —) and AIII (●, ---) in the presence of propranolol on R_T, E_T, and MABP in artificially-ventilated urethane-anesthetized animals. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals.

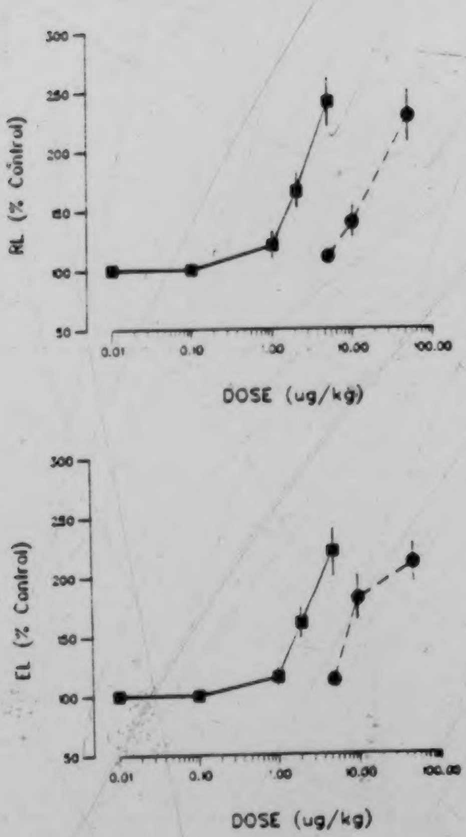

Figure 8-8: Effects of AII (■ , ——) and AIII (●, ----) in the presence of propranolol on R_L, E_L, and MABP in freely-respiring urethane-anesthetized animals. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals.

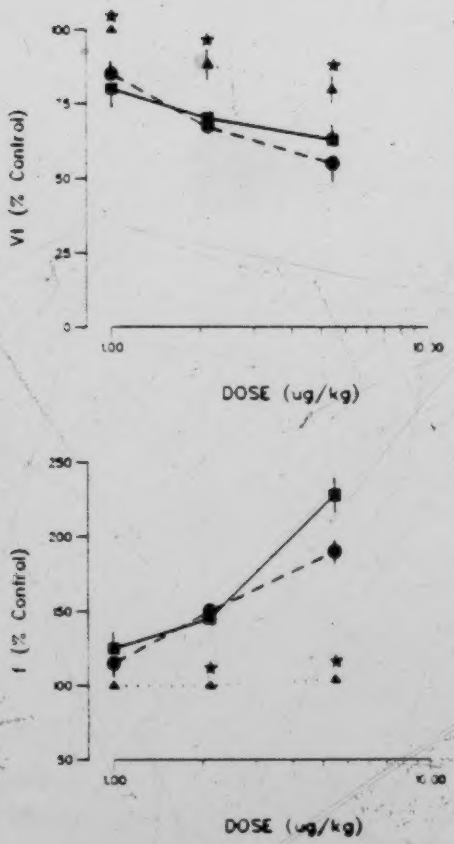

Figure 8-9: Effects of AII in the presence of propranolol on V_t and f in the intact animal (■, ——), after ADN and CSN section (●, ----), and bilateral vagotomy + ADN and CSN section (▲, ······) in freely-respiring urethane-anesthetized guinea pigs. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals. ★Statistically significant $p<0.05$.

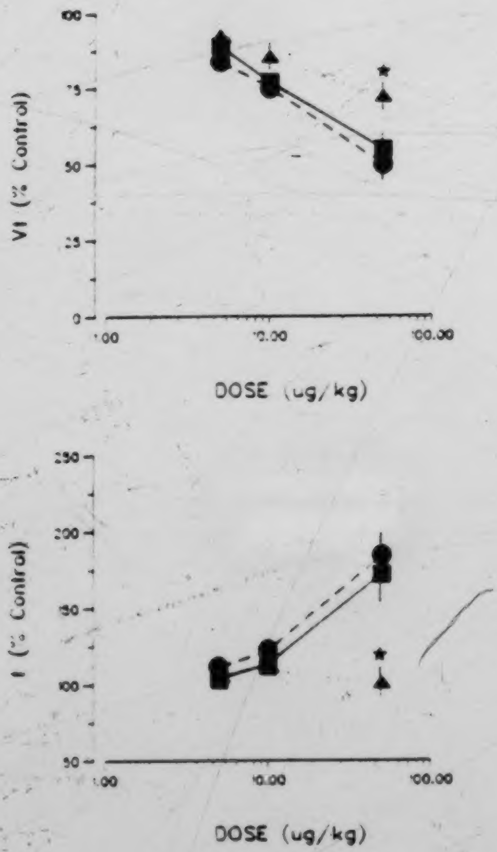

Figure 8-10: Effects of AIII in the presence of propranolol on $\bar{V}_t$ and f in the intact animal (■, ——), after ADN and CSN section (●, ----), and bilateral vagotomy + ADN and CSN section (▲, ······) in freely-respiring urethane-anesthetized guinea pigs. Baseline values are summarized in Table 8.1. Each point represents the mean of 4 animals. Statistically significant $p<0.05$.

CHAPTER IX

Effects of α-adrenoceptor stimulation on airway smooth muscle of normal and ovalbumin-sensitized guinea pigs *in vivo*

Introduction

Drug-induced bronchoconstriction in the guinea pigs is mediated via CSN afferents and by a non-vagal, presumably sympathetic system (41). A similar reflex response was claimed in response to unilateral CSN stimulation (44). While I was unable to repeat the latter study, I did show that selective CCr stimulation does increase bronchomotor tone by an unknown mechanism.

In the present study, I set out to determine whether sympathomimetics could induce bronchoconstriction in guinea pigs. I focussed primarily on the effects of α-adrenoceptor stimulation. The role of α-adrenoceptors in airway function is poorly understood and controversial (13, 100). α-adrenoceptor stimulation in the airways is claimed to induce bronchospasm in man and in animals via direct actions on airway smooth muscle (23). α-adrenoceptors also regulate transmitter release at cholinergic nerve endings, and thus may affect contraction of airway smooth muscle indirectly (106). In asthmatic patients, α-adrenoceptor function is altered, and it has been proposed that an important cause of asthma is an increase in bronchial α-adrenoceptor activity. Radioligand binding studies demonstrated the presence of α-adrenoceptors in the airways, and that the ratio of α- to β-adrenoceptors is increased in airways of asthmatic patients (246). Some functional studies claim that α-adrenoceptor activity is increased in diseased airways (146), others found that the effects of α-adrenoceptor stimulation on the airways of normal and asthmatic patients were identical (100).

The aim of the study was to examine the role of α-adrenoceptor stimulation in maintaining the airway caliber in normal guinea pigs.

The effects of sympathetic stimulation in ovalbumin-sensitized guinea pigs - an animal model of asthma - were also studied to determine whether they were altered in sensitized animals. In addition, the effects of β-adrenoceptor blockade were studied to determine if a withdrawal of sympathetic tone could alter airway patency.

Results

Baseline values for R_T, E_T, MABP, and HR determined in ovalbumin-sensitized, unsensitized control, untreated control, and chemically-sympathectomized animals are shown in Table 9-1. Values of R_T and E_T were greater ($p<0.05$) in ovalbumin-sensitized animals than in saline-treated controls. Reserpine-treated animals had lower MABP ($p<0.05$) and HR ($p<0.05$) than untreated controls, and their mean values for R_T and E_T were greater ($p<0.05$). 6-Hydroxydopamine had little effect on MABP, HR, R_T, or E_T.

i) Effects of α-adrenoceptor stimulants in untreated control guinea pigs.

Noradrenaline (NA) (0.1-20.0 μg/kg, i.v.) induced dose-dependent increases in E_T, MABP, and HR, and decreased R_T (Fig. 9-1). After animals were treated with propranolol (2.0 mg/kg, i.v.), NA induced dose-dependent increases in R_T (Fig. 9-1). Propranolol potentiated NA-induced increases in E_T, blocked the actions on NA on HR, and had no effect on MABP responses to NA (Fig. 9-1). Changes in R_T and E_T in response to NA returned to baseline within 2 min of administering NA, whereas increases in MABP and HR returned to baseline in 5-8 min, depending on the size of the dose of NA administered. Increases in E_T

before (r=0.68, n=16) and after (r=0.51, n=83) administering propranolol were correlated with increases in MABP. Also, the decreases (r=-0.73, n=16) and the increases (r=0.53, n=83) in R_T induced by NA in the absence and in the presence of propranolol, respectively, were correlated with increases in MABP.

In untreated control animals, phenylephrine (PE) (1.0-100.0 μg/kg, i.v.) induced dose-dependent increases in E_T and MABP, and decreased R_T and HR (Fig. 9-2). After treatment with propranolol (2.0 mg/kg, i.v.), PE had no effect R_T ($p \geq 0.50$). Propranolol did not alter the PE-induced increases in E_T and MABP and abolished the effects of PE on HR (Fig. 9-2).

In untreated control animals, nordefrin (1.0-100.0 μg/kg, i.v.) increased E_T, and MABP, but had no effect on R_T (Fig. 9-3). In the presence of propranolol, nordefrin had no statistically significant effects on R_T (p=0.42). Propranolol did not alter the E_T and MABP responses to nordefrin (p=0.35) (Fig.9-3). In the absence of propranolol, nordefrin increased HR in 3 of 7 animals, and decreased it in 4 of 7. Propranolol abolished the effects of nordefrin on HR (Fig.9-3).

The effects of the α-adrenoceptor stimulation on R_T and E_T were small or absent. Noradrenaline was the most potent and PE the least potent of the three agonists on MABP, E_T, and R_T. Before and after propranolol administration, the slope of the log dose-response curves (for E_T as a percent of control) for even the most potent agent, NA, was much less than those for histamine (Fig. 9-4).

ii) Effects of nerve sections and autonomic blockers

These experiments were conducted in animals given propranolol (2.0

mg/kg, i.v.) to eliminate effects mediated via β-adrenoceptors. The effects of NA on R_T and E_T were unchanged by bilateral vagotomy, sectioning of ADN and CSN, or sectioning the ADN and CSN, and then performing bilateral vagotomy (Fig.9-5). The effects of NA on MABP were not significantly ($p=0.46$) altered by these nerve sectionings. In the presence of propranolol, yohimbine (0.5 mg/kg, i.v.) did not alter baseline values of R_T, E_T, MABP, and HR, and had no effect on the increases in R_T, E_T, and MABP induced by NA. Prazosin (0.1 mg/kg, i.v.), given after propranolol and yohimbine, decreased baseline MABP (from 60 ± 4 to 36 ± 4 mm Hg), but did not alter baseline values of R_T and E_T. In the presence of propranolol and yohimbine, prazosin abolished NA-induced increases in R_T and E_T, and reduced NA's effects on MABP (Fig. 9-6). In the presence of propranolol alone, prazosin (0.1 mg/kg, i.v.) had similar effects. The ganglionic blocker hexamethonium (10.0 mg/kg, i.v.) did not alter baseline values of R_T and E_T, or affect NA-induced increases in R_T and E_T. However, it reduced baseline MABP (from 67 ± 2 to 52 ± 2 mm Hg, $p=0.01$), and potentiated NA's pressor actions (Fig. 9-7). Desipramine (0.2 mg/kg, i.v.) had no effect on baseline values of R_T, E_T, MABP, and HR, and did not significantly affect NA-induced increases in R_T and E_T ($p=0.38$). The MABP response to NA was potentiated by desipramine ($p<0.05$) (Fig. 9-8).

iii) Effects of chemical sympathectomies

In animals given reserpine (2.5 mg/kg, i.p.) 24 h before experiments, tyramine (0.25 mg/kg, i.v.) induced small (< 5.0 mm Hg) increases in MABP. In these animals, PE (Fig. 9-9) and nordefrin (Fig. 9-10) induced dose-dependent increases in E_T and MABP, and dose-dependent decreases in R_T. In reserpine-treated animals, the effects of PE and nordefrin on R_T and E_T were greater than in untreated animals. Propranolol (2.0 mg/kg, i.v.) potentiated the effects of both α-adrenoceptor agonists on E_T, but had no effect on MABP (Fig. 9-9, 9-10). In the presence of propranolol, PE and nordefrin had no statistically significant effect on R_T (Fig. 9-9, 9-10).

In guinea pigs given 6-hydroxydopamine (35.0 mg/kg, i.p.) 24 h before experiments, tyramine (0.25 mg/kg, i.v.) induced large increases in MABP (29 ± 4 mm Hg). In these animals, PE and nordefrin increased E_T and MABP, but had little effect on values of R_T. Propranolol did not modify any of these effects (Fig. 9-11).

iv) Effects of aerosol exposure in control and ovalbumin-sensitized guinea pigs

Control guinea pigs that had received 0.9 % saline (i.p.) showed no pulmonary responses to saline over the 14 days of exposure. By contrast, after a 1-2 min exposure to an aerosol containing 1% ovalbumin, animals that had received ovalbumin (i.p.) developed wheezing, coughing, dyspnea, and in extreme cases, cyanosis. Animals were removed immediately from the exposure chamber when they showed respiratory distress. Signs of respiratory distress gradually disappeared and animals recovered fully 20-30 min after exposure to ovalbumin aerosol. The exposure time required to induce the respiratory signs increased and the recovery period decreased over the 14 days of exposure to ovalbumin. In the course of an experiment, inflation pressure (intratracheal pressure) increased spontaneously periodically and returned to baseline within 5-10 min. These increases in intratracheal pressure during artificial ventilation were assumed to be a reflection of spontaneous bronchoconstriction.

v) Effects of adrenoceptor stimulants in control and ovalbumin-sensitized animals

In ovalbumin-sensitized animals, NA (0.1-20.0 ug/kg, i.v.) induced small increases in R_T and E_T, and dose-dependent increases in MABP. Propranolol (2.0 mg/kg, i.v.) potentiated the actions of NA on R_T; however, it did not alter the effects of NA on E_T and MABP (Fig. 9-12). In the presence of propranolol, prazosin (0.1 mg/kg, i.v.) abolished NA-induced increases in E_T, and reduced the increases in R_T and MABP (Fig. 9-13). Increases in R_T and E_T induced by NA in the presence of propranolol were unchanged after bilateral vagotomy followed by sectioning of the ADN and CSN, whereas MABP responses to NA were greater (Fig. 9-14). In saline-treated control animals, effects of NA were similar to those seen in the untreated group (Fig. 9-15). In comparison with unsensitized animals, increases in R_T and E_T induced by histamine (i.v.) were significantly greater ($p<0.05$) in ovalbumin-sensitized animals (Fig. 9-16). Although NA induced larger increases in R_T, the slope of the dose-response line was still less than that for histamine.

vi) Effects of propranolol in untreated, chemically-sympathectomized, and ovalbumin-sensitized guinea pigs

In untreated and chemically-sympathectomized guinea pigs, propranolol (2.0 mg/kg, i.v.) had little effect on baseline values of R_T and E_T. By contrast, in ovalbumin-sensitized animals, the same dose of propranolol increased baseline values of R_T, without affecting values of E_T (Fig. 9-16). The increases in R_T persisted for the duration of the experiment. Propranolol reduced HR (from 253 ± 8 to 196 ± 6 beats/min) consistently and induced a short-lived fall in MABP in all three groups of animals.

Discussion

The aim of this study was to examine the role of α-adrenoceptors in the regulation of airway caliber. Experiments were carried out in normal, healthy guinea pigs and in ovalbumin-sensitized guinea pigs. The effects of α-adrenoceptor stimulation are complex and differ between unsensitized and ovalbumin-sensitized guinea pigs. The effects of α-adrenoceptor stimulation on unsensitized and ovalbumin-sensitized guinea pigs will be considered separately to simplify the discussion.

Unsensitized Animals

The role of α-adrenoceptor in regulation of airway patency in healthy patients and normal animals is controversial. In vivo, α-adrenoceptor agonists are reported to dilate and constrict human airways (230). In guinea pigs, experiments in vitro have shown that α_1-adrenoceptor agonists constrict peripheral lung strips (5). In the same species, studies in vivo have shown that NA in the presence of propranolol induces bronchoconstriction (148), whereas selective α_1-adrenoceptor agonists induce a propranolol-resistant bronchodilation (5). The present study was carried out to evaluate the effects of α-adrenoceptor stimulation in normal healthy guinea pigs. Three agonists were examined: 1) NA, an α- and β-adrenoceptor agonist; 2) Nordefrin, an α-adrenoceptor agonist with preferential α_2-adrenoceptor activity (266, 267); and 3) PE, an α_1-adrenoceptor agonist with weak β-adrenoceptor activity (266). Initially, all experiments were conducted in the absence of adrenoceptor blockers and without interruption of the reflex pathways. My results indicate that all three agonists are potent vasoconstrictors, but have weak bronchoconstrictor and bronchodilator actions - as evidenced by increases in E_T and falls in R_T.

The bronchodilator effects of all three agonists were apparently mediated via β-adrenoceptors. In the presence of propranolol, all three agonists either increased or had no effect on R_T. Only NA induced reproducible and dose-dependent increases in R_T. α_1-adrenoceptor stimulation is claimed to induce a propranolol-resistant bronchodilation in guinea-pig airways precontracted with histamine or ACh (5). My results indicate that propranolol-resistant bronchodilation is not present in the airways of untreated normal guinea pigs. Also, propranolol potentiated the increases in E_T induced by NA and nordefrin, but it did not significantly affect those to PE. These results demonstrate the need to study the effects of α-adrenoceptor stimulation on airway smooth muscle in the presence of β-blockade.

In dogs, the bronchospastic effects of NA are mediated by post-junctional α_2-adrenoceptors (31). By contrast, guinea-pig and human airways are reported not to have post-junctional α_2-adrenoceptors, but do contain pre-junctional α_2-adrenoceptors which modulate cholinergic transmission (106, 107). The bronchoconstrictor effects of NA are believed to be mediated via α_1-adrenoceptors (148). My results indicate that the bronchoconstrictor effects of α-adrenoceptor agonists in the presence of propranolol arise from a direct action on α_1-adrenoceptors.

Reflex pathways of the ADN, CSN, or vagus do not affect the NA-induced increases in R_T and E_T and sectioning these nerves was without effect. This, and the lack of effect of the ganglionic blocker hexamethonium, demonstrate that NA has a direct action. Yohimbine had no effect on the pulmonary and cardiovascular responses to NA, whereas prazosin abolished the increases in R_T and E_T, and significantly reduced the MABP response to NA. This demonstrates clearly that α_1-adrenoceptors mediate the bronchoconstriction induced by adrenoceptor agonists. Furthermore, desipramine did not affect the NA-induced increases in R_T and E_T. This suggests the receptors are not supplied by a functional adrenergic innervation to the airways and that uptake I is not important. However, whether uptake I inhibition will affect a response is dependent not only on the presence of adrenergic nerves, but also on the width of the junctional cleft (278). Thus, the inability of desipramine to potentiate the responses to NA does not exclude the possibility of a functional adrenergic innervation.

The effects of nordefrin and PE were studied in chemically-sympathectomized animals. In animals pretreated with 6-hydroxydopamine, tyramine induced significant increases in MABP. This effect of tyramine may be due to the release of catecholamines from the adrenal medulla, which is unaffected by 6-hydroxydopamine, but is depleted by reserpine. The differential effects of 6-hydroxydopamine and reserpine on baseline R_T, E_T, MABP, and HR may be explained by this distinction. The effects of nordefrin and PE in 6-hydroxy-dopamine treated animals, were not significantly different from those in the untreated group.

In reserpine-treated guinea pigs, tyramine had no effect on MABP, indicating a near-complete sympathectomy. Reserpine pretreatment reduced baseline MABP and HR, and increased baseline R_T and E_T. In reserpine-treated animals, the bronchodilator and bronchoconstrictor effects of PE and nordefrin were greater than in the untreated group. The increased bronchodilator activity of nordefrin and PE may be due to the increased baseline R_T. Propranolol had little effect on the bronchoconstrictor responses to nordefrin and PE, but abolished or reduced their bronchodilator actions.

In the absence and the presence of propranolol, the bronchoconstrictor effects of NA, PE, and nordefrin were reflected predominantly in an increase in E_T. This suggests that these agonists affect the small airways. Changes in large airway caliber are reflected by changes in R_T, whereas changes in small airway caliber are reflected by changes in R_T and E_T (14). If these agonists do act on small airways, then one could argue that E_T and R_T should increase to the same degree. In the presence of propranolol, all three agonists studied induced small increases in R_T, but only the effects of NA were significant and reproducible. Similar differential responses to drugs have been reported previously in this model using the least-squares-fitting technique to estimate R_T and E_T (98). This differential response may reflect the degree of change. In humans, the contribution of the small airways to R_T has been estimated to range from 20-50 % (14). To my knowledge, no one has yet studied the contribution of small airways to R_T in the guinea pig. However, it is clear that small airways contribute to only a fraction of R_T and a small decrease in their total cross section might not be detected in terms of a change in R_T.

Changes in the small airways can increase E_T by directly altering the distensibility of the lung parenchyma or by decreasing the amount of parenchyma participating in breathing; thus, E_T may be a more sensitive indicator of changes in small airway caliber (81).

The nature of the bronchoconstrictor response to α-adrenoceptor stimulation is unclear. It has been proposed that the bronchoconstrictor effects of NA are secondary to its action on the vasculature (73). All three of the agonists examined in this study are potent vasopressor agents and, in comparison with a bronchospastic agent like histamine, have very weak bronchospastic actions. As potent vasopressor agents, all three drugs increase pulmonary vascular pressure. Increases in pulmonary vascular pressure lead to congestion which is reflected in an increase in the resistance of small airways (73). Adrenaline has been shown to constrict peripheral lung strips by contracting airway and vascular smooth muscle, and contractile elements in the interstitium, but it dilates bronchi (84). Moreover, it has been reported that, in vitro, the contractile effects of α_1-adrenoceptor agonists were greater than those of ACh (5). The elevated apparent bronchoconstrictor activity of α_1-adrenoceptor agonists in vitro is postulated to be due to their actions on the other contractile elements in the lung (5, 84). Others have proposed that NA, acts directly on α_1-adrenoceptors located on airway smooth muscle (148). This mechanism assumes that α_1-adrenoceptors are located preferentially in the small airways. Binding studies have shown that although α-adrenoceptors are scarce in the airways, their numbers do increase as the diameter of the airways decreases (25, 26). The scarcity of α-adrenoceptors explains the small size of the bronchoconstrictor actions α-adrenoceptor agonists and the sensitivity of this response to prazosin, and suggests that these receptors do not play a significant role in the regulation of airway caliber in healthy animals. As pulmonary blood pressure was not monitored in this study, and I am unable to exclude one explanation in favor of the other.

Ovalbumin-sensitized guinea pigs

It has been proposed that the bronchial hyperreactivity in asthma is associated with increased α-adrenoceptor activity coupled with a diminished β-adrenergic function (245). Binding studies have shown that the ratio of α- to β-adrenoceptors in normal lungs is less than that in asthmatic lungs (246). Similar findings have been reported in ovalbumin-sensitized guinea pigs chronically exposed to ovalbumin aerosol (25, 95, 172). Although some studies claim that α_1-agonists constrict asthmatic airways (146, 235), others were unable to confirm these findings (100, 251). The present study was conducted to examine the effects of adrenoceptor stimulation in ovalbumin-sensitized guinea pigs.

The use of ovalbumin-sensitized guinea pigs chronically exposed to ovalbumin aerosol requires some qualification. Ideally, animal models of asthma should exhibit the features of asthma. No single animal model presents all the features of bronchial asthma. Although ovalbumin-sensitized guinea pigs chronically exposed to ovalbumin aerosol do not present bronchial asthma, they do show a number of the features associated with asthma. These animals exhibit non-specific hyperresponsiveness and physiological responses to antigen challenge resembling those in man (261). The immunological pathways leading to bronchospasm are similar to those in allergic asthma (140). However, the pathological lesions of asthma are not completely reproduced in these animals (140, 197). It is also claimed that ovalbumin-sensitized guinea pigs do not show the spontaneous persistent bronchoconstriction typically featured in asthma, but my results demonstrate that ovalbumin-sensitized guinea-pig airways do constrict spontaneously. My results demonstrate that ovalbumin-sensitized guinea pigs - like asthmatics (274) - show resting β-adrenoceptor bronchodilator tone and a hypersensitivity to histamine not found in normal humans or unsensitized guinea pigs. Finally, chronic exposure of ovalbumin-sensitized guinea pigs to ovalbumin aerosol decreases small airway caliber - as evidenced by the high baseline E_T.

In ovalbumin-sensitized guinea pigs, NA (i.v.) induced dose-dependent increases in R_T and E_T. Propranolol exacerbated the R_T response, but had little effect on the increases in E_T induced by NA. Chemical sympathectomy was not performed in ovalbumin-sensitized guinea pigs. The bronchoconstrictor effects of NA appear to arise from a direct action on α_1-adrenoceptors. They were unaffected by sectioning of the ADN, CSN, and vagi, but were abolished or significantly reduced by prazosin.

The bronchoconstrictor actions of NA appear to differ from those in normal animals because they were reflected primarily as a change in R_T. This suggests that the bronchoconstrictor effects of NA in this animal model are predominantly on the large airways. However, the lack of a large increase in E_T in response to NA, may be an artifact of the high baseline E_T in ovalbumin-sensitized guinea pigs. The actions of NA may still be predominantly on the small airways and the expected increase in E_T may be concealed by the high baseline. It is noteworthy that in ovalbumin-sensitized animals, the increases in R_T induced by histamine were also much greater than the E_T responses, whereas in unsensitized animals they were equivalent.

The lack of a bronchodilator response to NA in ovalbumin-sensitized guinea pigs suggests that β-adrenoceptor function is diminished in these animals. A number of groups have reported that chronic exposure of ovalbumin-sensitized guinea pigs to ovalbumin aerosol decreases β-adrenoceptor ligand binding in the airways (25, 95, 172). Animals sensitized to ovalbumin, but not chronically exposed to ovalbumin aerosol show only a small decrease in β-adrenoceptor ligand binding (95) and do not show reduced bronchodilator responses (55). This suggests that the decrease in the number of β-adrenoceptors and in β-adrenergic bronchodilator responses are not a direct effect of ovalbumin-sensitization, but an effect of the increases bronchodilator tone induced by chronic exposure to nebulized ovalbumin. Presumably this bronchodilator tone is mediated by the release of circulating catecholamines. The bronchoconstrictor response to propranolol in these animals may be indicative of this tone.

In ovalbumin-sensitized guinea pigs, the bronchoconstrictor effects of NA administration in the presence of propranolol were much greater than in unsensitized animals. This suggests an increase in the number or in the activity of α-adrenoceptors. Binding studies have shown that the number of α-adrenoceptors is increased by chronic exposure of ovalbumin-sensitized guinea pigs to ovalbumin aerosol (25, 172). By contrast, in vitro, the actions of NA on guinea pig trachea were not altered by ovalbumin-sensitization (H.S. Shin, personal communication). This may reflect the many differences between trachea and the airways in this species; however, Taylor et al. (256) claim that the effects, in vitro, of NA on peripheral lung strips are not altered by chronic exposure to ovalbumin aerosol and suggest that the increase in α-adrenoceptor binding is not correlated to an increase in function.

The differences between my findings and those of Turner et al. (256) may very well be explained by mechanics of airway contraction. Airways are narrowed by smooth muscle spasm, swelling of submucosal connective tissue, and exudate on the surface of the lumen (175). Hogg et al. (123) have recently shown that in vivo hyperresponsiveness to histamine in smoke-exposed guinea pigs is due to normal muscle shortening acting in concert with airway walls thickened by inflammatory swelling. Airway smooth muscle is arranged in a geodesic pattern. Contraction of this muscle narrows and shortens the airway and, in doing so, it folds and distorts the mucosa further reducing the caliber of the airway. When the mucosa is inflamed or thickened, the same degree of smooth muscle contraction may produce a greater decrease in airway caliber than normal. This suggests that ovalbumin-sensitization may not increase the smooth muscle response to NA but, may still potentiate its effects on airway patency. Clearly, this is speculative on my part as I have not demonstrated that ovalbumin challenge does thicken the airway wall by swelling, but presumably the release of inflammatory mediators by recurrent antigen challenge should cause inflammation. It is of interest to note that, in most species, α_1-adrenoceptor-mediated bronchoconstriction is only apparent when the airways are precontracted by inflammatory mediators, or in response to antigen challenge (22). Although this response has been ascribed to an "unmasking" of α-adrenoceptors, mucosal inflammation could also explain the phenomenon.

The increased sensitivity to histamine in the ovalbumin-sensitized animal is similar to that reported in smoke-exposed guinea pigs by Hogg and his colleagues and may also be due to increased mucosal inflammation. It is possible that the bronchoconstriction induced by propranolol in the ovalbumin-sensitized guinea pig may be explained by mucosal thickening. However, mucosal thickening potentiates the constrictor response to a given agent, and the airways appear hyperresponsive. In normal animals propranolol had no effect on airway patency due to the absence of sympathetic bronchodilator activity. Thus, regardless of any potentiation by mucosal inflammation, the constrictor response to propranolol still implies that a sympathetic bronchodilator tone is present in ovalbumin-sensitized animals.

In conclusion, NA and α-adrenoceptor agonists have little effect on airway patency in normal animals. The effects of NA on airway patency is potentiated in ovalbumin-sensitized animals. This effect does not appear to be due to an increase in the sensitivity of airway smooth muscle to NA, but may reflect the non-specific hyperresponsiveness of the airways in this animal model of asthma.

Table 9-1. Summary of the mean (± s.e.m.) values of thoracic resistance (R_T), elastance (E_T), mean arterial blood pressure (MABP), and heart rate (HR) in normal and treated guinea pigs anesthetized with α-chloralose.

GROUP	n	R_T cmH$_2$O/s/mL	E_T cm/mL	MABP mm Hg	HR beats/min
Untreated	40	0.367±0.025	1.415±0.105	67±2	256±1
Ovalbumin	7	0.419±0.064*	2.881±0.186*	64±3	280±10
Saline	4	0.347±0.028	1.355±0.094	59±6	260±10
Reserpine	8	0.430±0.044*	1.619±0.104*	46±4*	180± 3*
6-Hydroxy-dopamine	5	0.372±0.023	1.451±0.701	65±4	260± 8

* $p<0.05$ significantly different from that in untreated or saline-treated α-chloralose anesthetized, artificially ventilated animals.

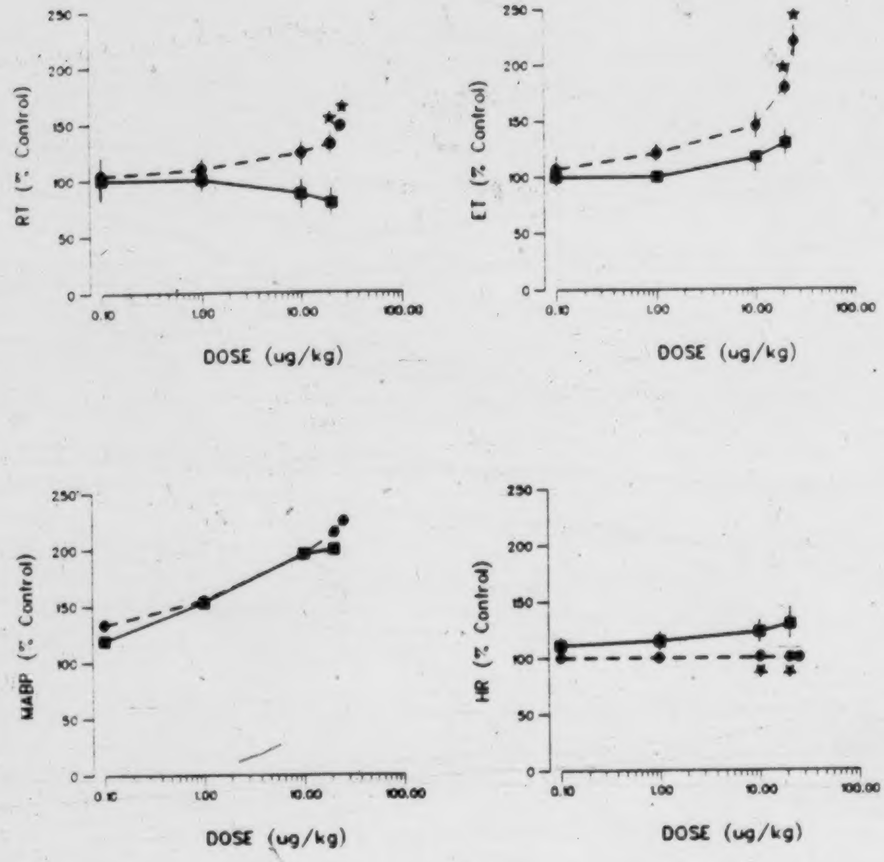

Figure 9-1: Effects of noradrenaline on R_T, E_T, MABP, and HR before (■, —) and after (●, ---) propranolol (2.0 mg/kg, i.v.) in 4 untreated animals anesthetized with α-chloralose. Baseline values are summarized in Table 9.1. Each point represents the mean ± s.e.m. ★Statistically significant $p<0.05$.

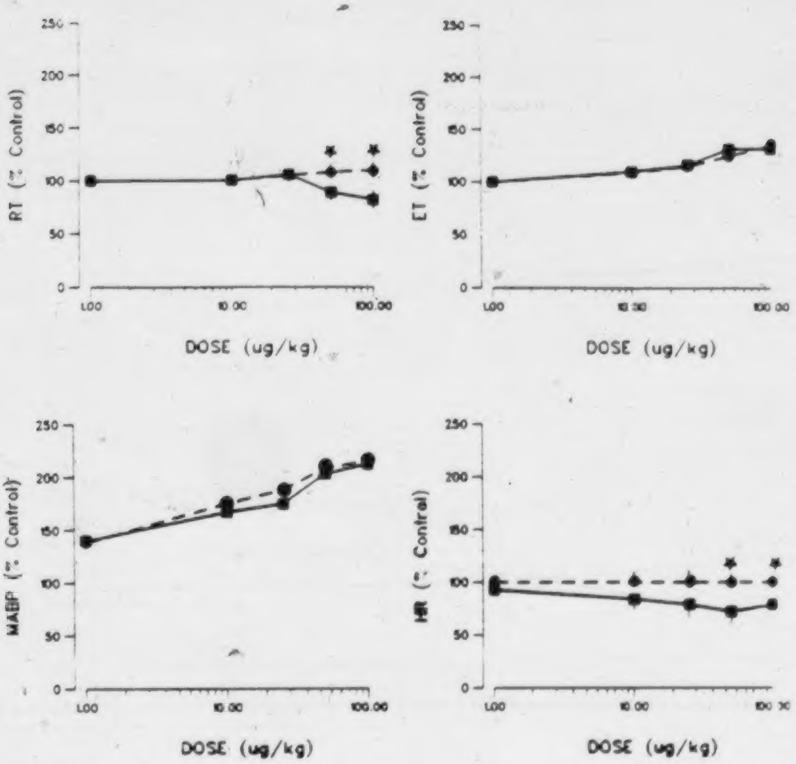

Figure 9-2: Effects of phenylephrine on R_T, E_T, MABP, and HR before (■,——) and after (●, ---) propranolol (2.0 mg/kg, i.v.) in 4 untreated animals anesthetized with α-chloralose. Baseline values are summarized in Table 9-1. Each point represents the mean ± s.e.m. ★Statistically significant $p<0.05$

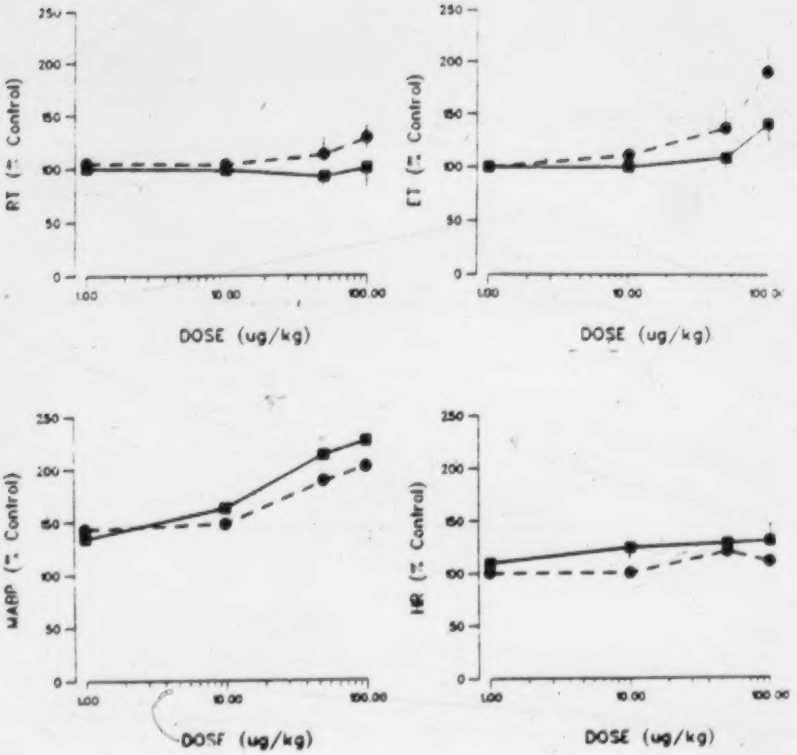

Figure 9-3: Effects of nordefrin on R_T, E_T, MABP, and HR before (■, —) and after (●, ---) propranolol (2.0 mg/kg, i.v.) in 4 untreated animals anesthetized with α-chloralose. Each point represents the mean ± s.e.m.

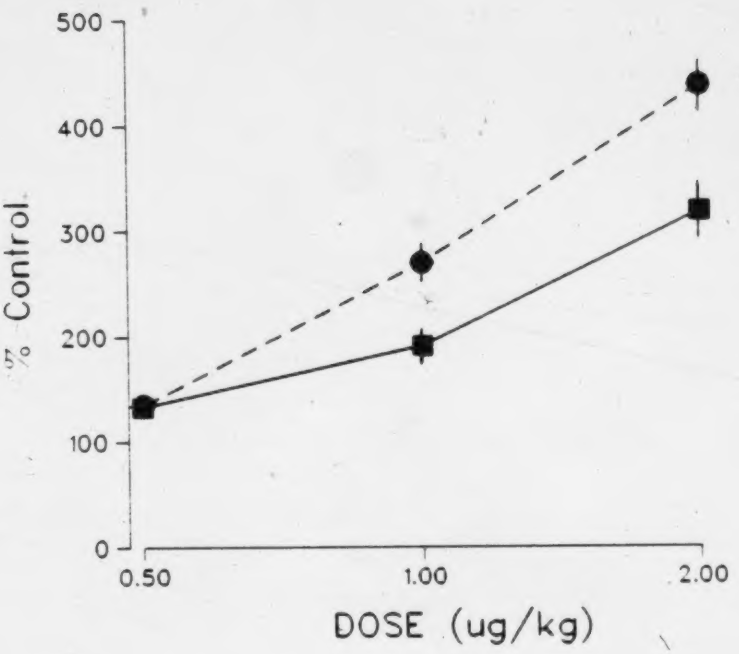

Figure 9-4: Effects of histamine on R_T (■, ——) and E_T (●, ---) in 4 saline-treated guinea pigs anesthetized with α-chloralose. Each point represents the mean ± s.e.m.

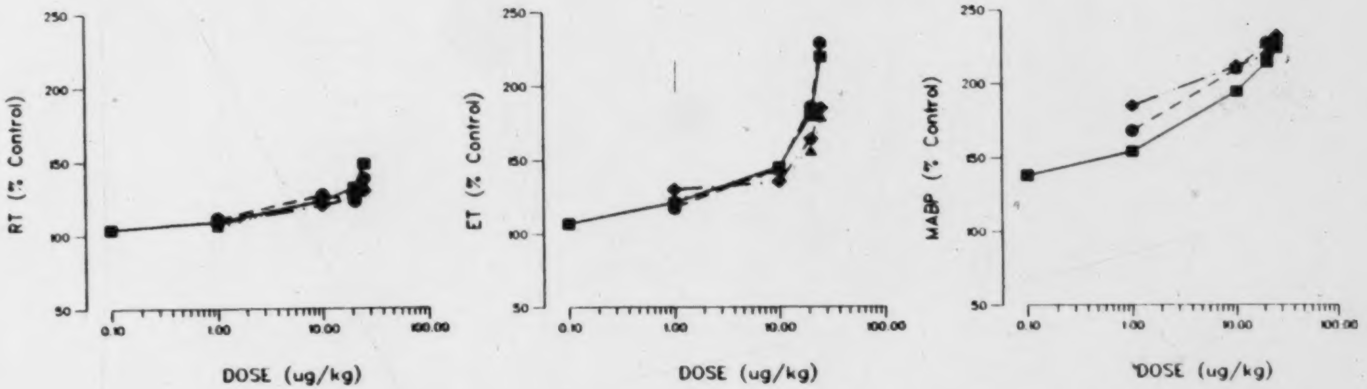

Figure 9-5: Effects of nerve sections on the R_T, E_T, MABP responses to noradrenaline in the presence of propranolol. Control (■ , ——), bilateral vagotomy (● , ---), section of the ADN and CSN (◆ , —·—), and bilateral vagotomy followed by section of the ADN and CSN (▲ , ····). Each point represents the mean ± s.e.m. of 4 animals anesthetized with α-chloralose.

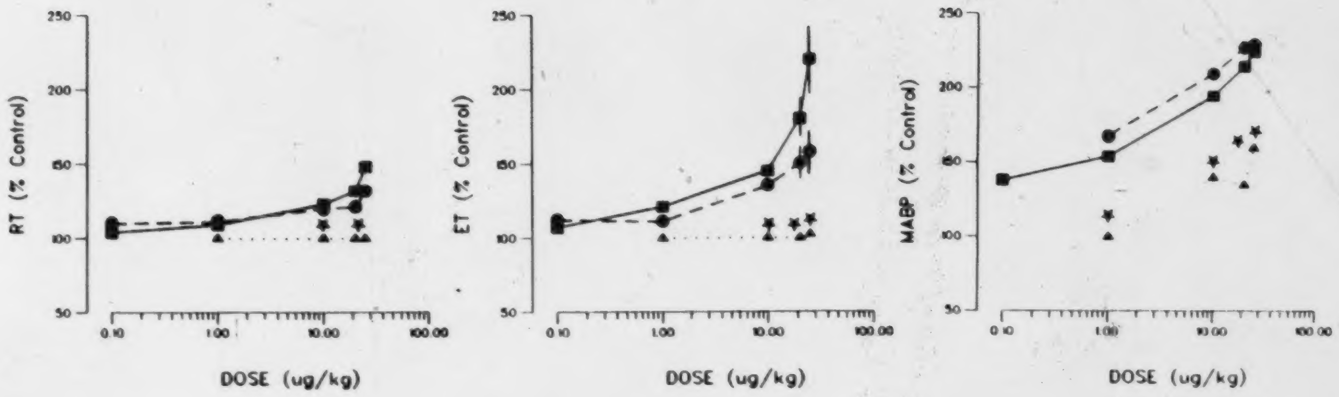

Figure 9-6: Effects of noradrenaline on R_T, E_T, and MABP in the presence of propranolol (2.0 mg/kg, i.v.)(■, ——), propranolol and yohimbine (0.5 mg/kg, i.v.) (●, ---), and propranolol, yohimbine, and prazosin (0.1 mg/kg, i.v.) (▲, ····). Each point represents the mean ± s.e.m. of 5 animals anesthetized with α-chloralose.

★Statistically significant $p<0.05$

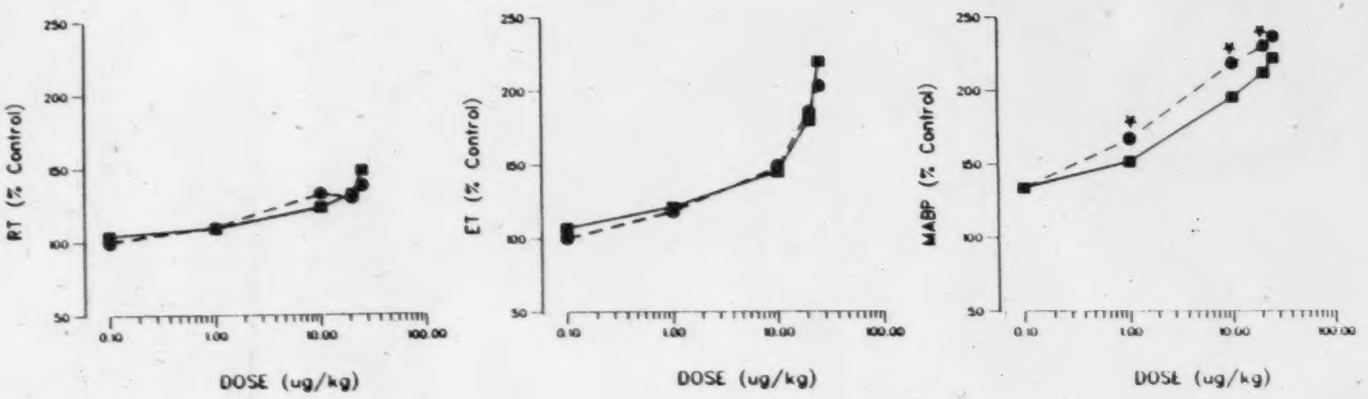

Figure 9-7: Effects of noradrenaline in the presence of propranolol on R_T, E_T, and MABP before (■, ——) and after (●, ---) hexamethonium (10 mg/kg, i.v.). Each point represents the mean ± s.e.m. of 4 animals anesthetized with α-chloralose.

✶Statistically significant $p<0.05$

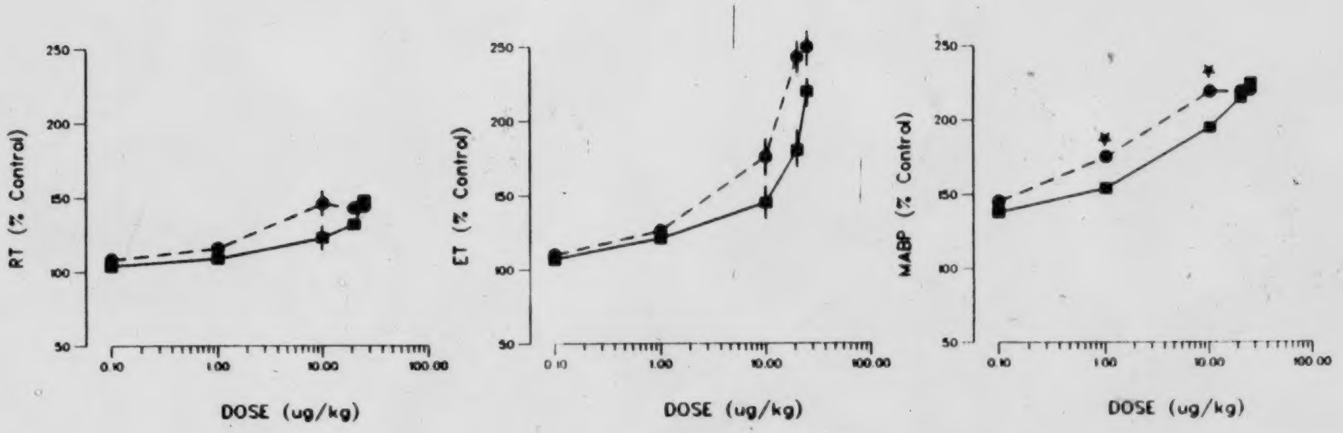

Figure 9-8: Effects of noradrenaline in the presence of propranolol on R_T, E_T, and MABP before (■, ——) and after (●, ---) desipramine (0.2 mg/kg, i.v.). Each point represents the mean ± s.e.m. of 4 animals anesthetized with α-chloralose.

★Statistically significant $p<0.05$

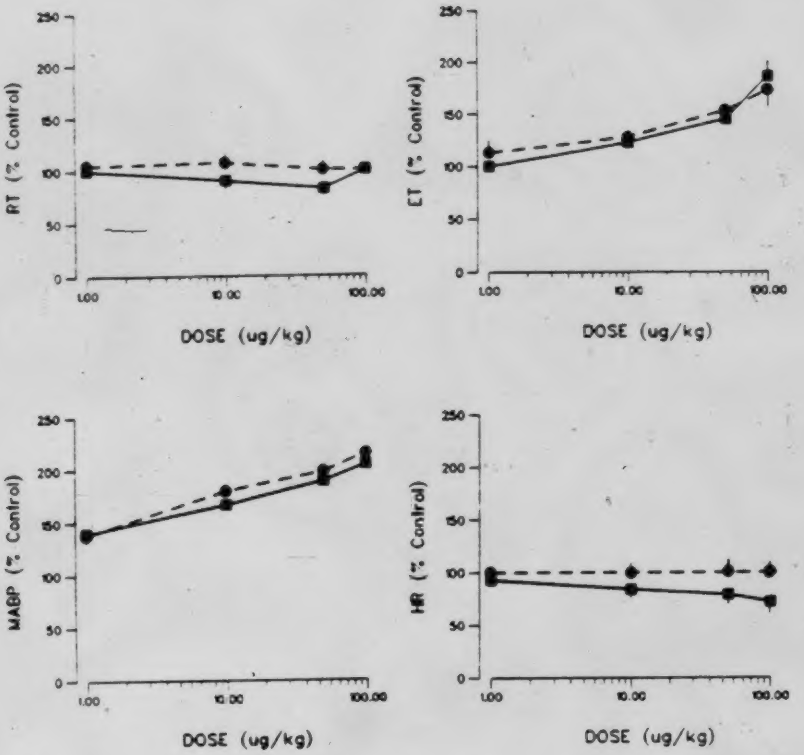

Figure 9-9: Effects of phenylephrine on R_T, E_T, MABP, and HR before (■, —) and after propranolol (●, ---) in 5 animals pretreated with reserpine and anesthetized with α-chloralose. Baseline values are summarized in Table 9-1. Each point represents the mean ± s.e.m.

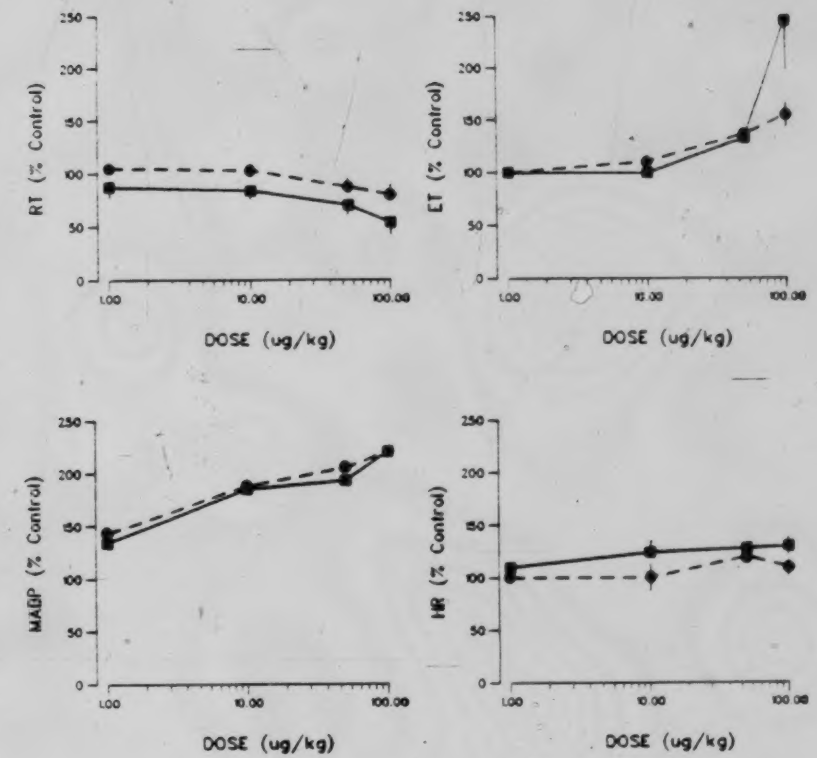

Figure 9-10: Effects of nordefrin on R_T, E_T, MABP, and HR before (■, —) and after propranolol (●, ---) in 5 animals pretreated with reserpine and anesthetized with α-chloralose. Baseline values are summarized in Table 9-1. Each point represents the mean ± s.e.m.

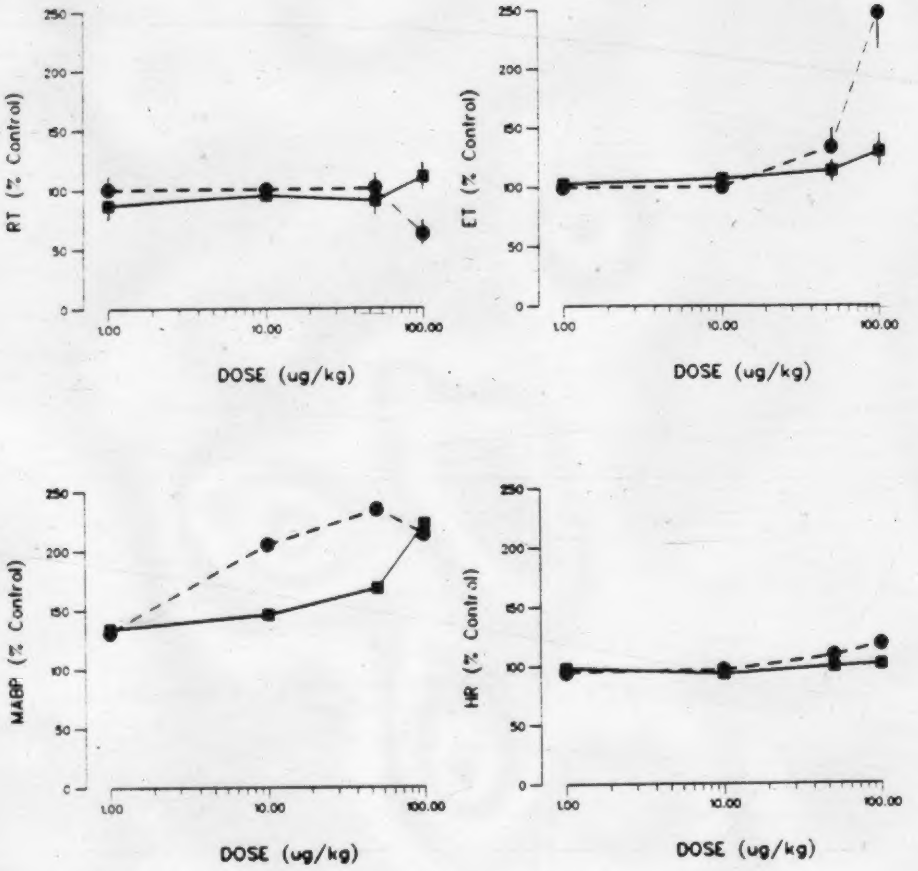

Figure 9-11: Effects of nordefrin (●, ---) and phenylephrine (■, ——) on R_T, E_T, and MABP in 5 animals pretreated 6-hydroxydopamine and anesthetized with α-chloralose. Baseline values are summarized in Table 9-1. Each point represents the mean ± s.e.m.

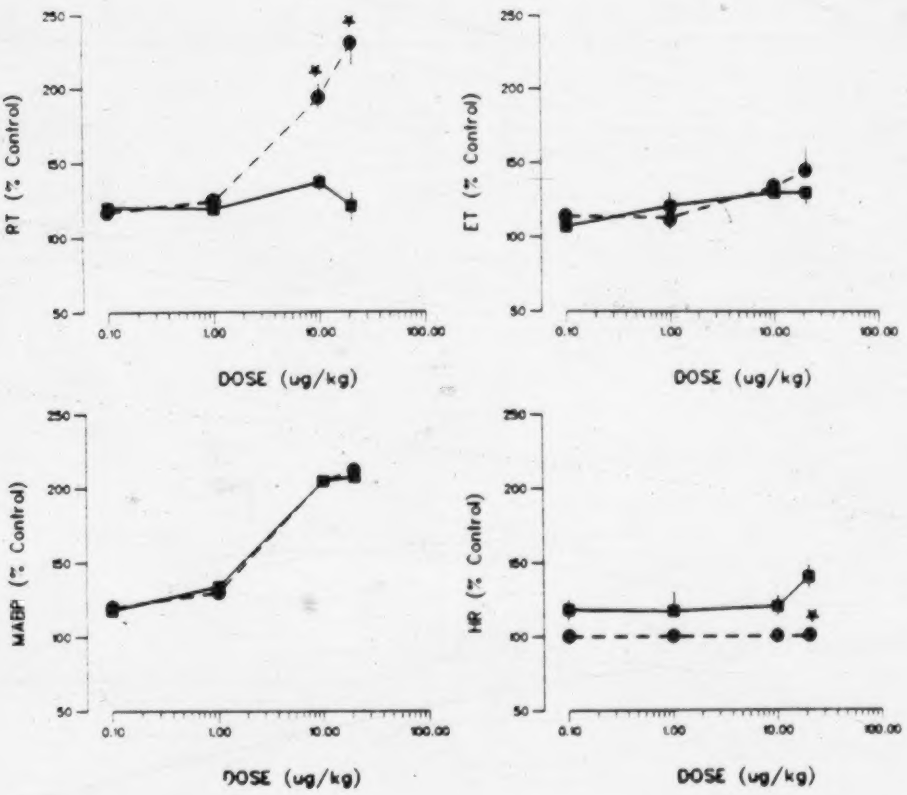

Figure 9-12: Effects of noradrenaline on R_T, E_T, MABP, and HR before (■, —) and after (●, ---) propranolol (2.0 mg/kg, i.v.) in 4 ovalbumin-sensitized animals anesthetized with α-chloralose. Baseline values are summarized in Table 9-1. Each point represents the mean ± s.e.m.

★Statistically significant $p<0.05$

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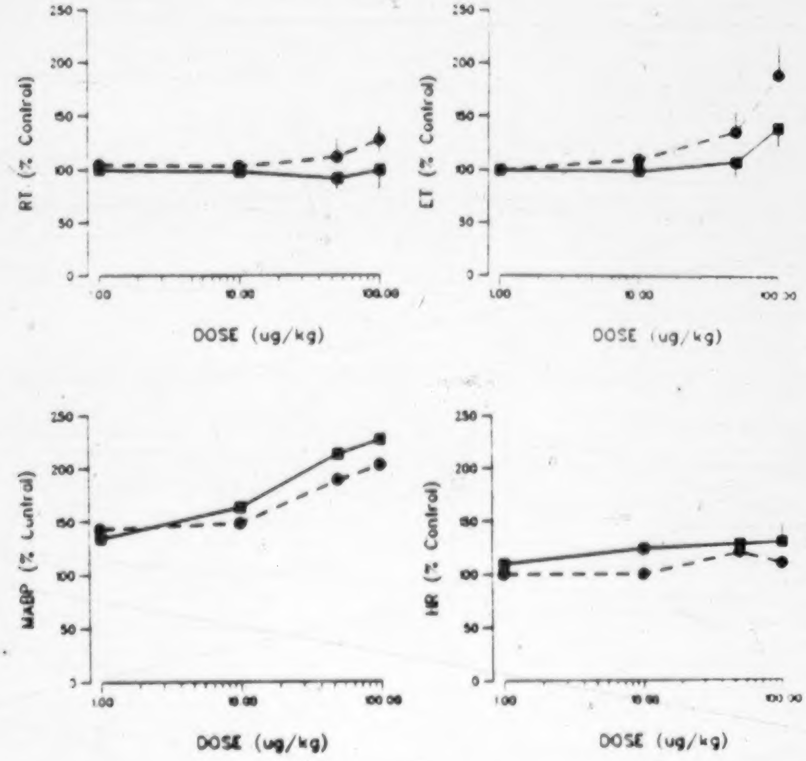

Figure 9-15: Effects of noradrenaline on R_T, E_T, MABP, and HR before (■, —) and after (●, ---) propranolol (2.0 mg/kg, i.v.) in 4 saline-treated animals anesthetized with α-chloralose. Each point represents the mean ± s.e.m.

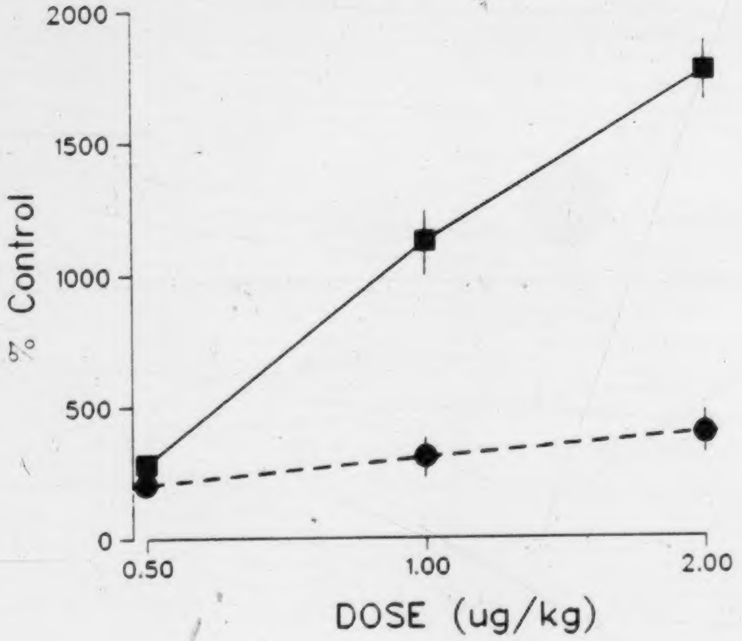

Figure 9-16: Effects of histamine on R_T (■, —) and E_T (●, ---) in 4 ovalbumin-treated guinea pigs anesthetized with α-chloralose. Each point represents the mean ± s.e.m.

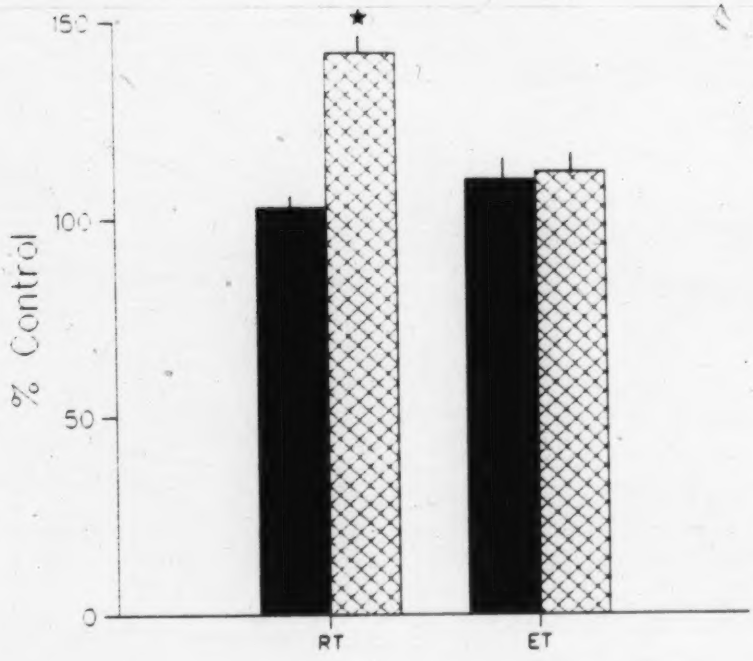

Figure 9-17: The effects of propranolol on R_T and E_T in untreated animals (solid bars) and ovalbumin-sensitized guinea pigs (hatched bars) anesthetized with α-chloralose. ★ Statistically significant $p<0.05$.

CHAPTER X

Discussion and Summary

Earlier work in this laboratory indicated that CSN afferents and sympathetic efferents play a role in the maintenance of airway patency in guinea pigs. It also suggested that NSAID and TZ, agents which elicit bronchoconstriction may act via CSN afferent and sympathetic efferents in guinea pigs. The present study was undertaken to clarify the roles of CSN afferents and sympathetic nerve efferents on airway function in guinea pigs. Before discussing the relevance of the findings, it is necessary to comment on their validity and their reliability.

Critique of the Methods

I report here the successful development of a method of recording nerve traffic in CSN and the development of a vascularly-isolated sinus preparation. To my knowledge, this is the first time CSN afferents and the reflexes they mediate have been thoroughly studied in guinea pigs. One of the problems with this animal model is that the CSN is very fine. This precluded the study of single-unit CBr or CCr fibers or homogeneous bundles of afferents. Furthermore, in this species, the carotid sinus and body are closely apposed and are not amenable to surgical separation, making it difficult to assign the action of a given agent to a particular receptor type.

These limitations also precluded the study of chemoreflexes and baroreflexes in isolation from each other. A similar problem is found in other species, such as the cat (50). When studying baroreflexes in these species, chemoreceptor activity is removed by allowing the animal to breathe freely O_2 or O_2-enriched air. The sinus may be perfused with either the animal's own blood or with an oxygenated Krebs solution. Oxygen was supplied to the guinea pigs at all times during the baroreflex studies reported here. This procedure requires monitoring of arterial blood gases and pH. Although $P_{et}CO_2$ was monitored in freely-respiring animals, a continuous record of these data was not kept, and blood gases and pH were not measured. Thus, I am unable to provide any data which demonstrate unequivocally that chemoreceptors were not stimulated in the free-respiring animal. Chemo- and baroreflexes can interact either positively or negatively (2). However, in the freely-respiring urethane-anesthetized group, there appeared to be little change in chemoreceptor activity in the course of an experiment- as evidenced by a lack of a significant change in f or V_t. Both V_t and f did fluctuate in freely-respiring animals anesthetized with pentobarbital, but this reflected the level of anesthesia. The problem may be of less magnitude in artificially-ventilated guinea pigs, in which $P_{et}CO_2$ was maintained at 4.5 % by adjusting $\dot{V}_e$. Nevertheless, in future studies, blood gases and pH should be carefully monitored.

Finally, a note on the lack of dose-response curves with a full range of doses. In neurophysiological studies, the number of doses tested was restricted by the length of time that the preparation remained stable, the doses of ASA, IND, and TZ were limited to a small range chosen on the basis of their effectiveness in eliciting bronchoconstriction. In examining the effects on airway patency of AII and sympathomimetics, a narrow range of doses was studied due to the pressor actions of these drugs. The drugs used in these studies were more potent pressor than bronchoconstrictor agents. The threshold bronchoconstrictor doses produced severe vasoconstriction, which precluded increasing the doses further. Thus, while a large range of doses was tested, maximal or near maximal doses for the bronchoconstrictor responses may not have been attained.

Summary and Relevance of the Findings

As stated above the purpose of this thesis was to clarify the role of CSN afferents and sympathetic efferents on airway function in guinea pigs. With this as a framework, I first sought to determine whether ASA, IND and TZ altered CSN activity and to establish if this action was limited to either CBr or CCr.

In the neurophysiological studies, ASA, IND, and TZ (i.v.) increased CSN activity, probably by an action on CBr. To examine this more directly, the effects of ASA and TZ on CSN activity were tested using a vascularly-isolated sinus preparation, in which only TZ was shown to increase CBr activity. The mechanism of action of this drug on CBr was not elucidated. A non-specific effect was suggested because TZ-induced increases in CBr sensitivity were only present at high concentrations of the drug.

The relevance of my findings to ASA idiosyncrasy in asthmatics is unclear. Recent reports in the literature indicate that cross-reactivity to TZ in the sub-population of asthmatics sensitive to ASA was grossly overestimated (243, 244). It has been suggested that the anti-cyclooxygenase action of the NSAID may be the cause of the idiosyncratic hypersensitivity to these drugs. In light of this evidence, it is clear that effects of PG on CSN activity should be clarified, especially with respect to the mechanism of ASA- and IND-induced increases in CSN activity. This may be accomplished by perfusing arachidonic acid through the vascularly-isolated sinus to stimulate PG synthesis or, alternatively, using more effective - and more stable and soluble - cyclooxygenase inhibitors to block PG synthesis. However, these studies would face similar problems to those I encountered: 1) the inability to isolate CBr from CCr fibers; 2) the technical difficulties of isolating and maintaining the perfused sinus preparation, while recording from the CSN in this species.

The findings of the neurophysiological studies indicated that ASA, IND and TZ acted on CBr. The effects of CBr stimulation on airway patency were examined by electrical stimulation of CSN and by increasing ISP to selectively stimulate CBr. In contrast to the earlier work (201), stimulation of CSN failed to alter airway patency, although a fall in MABP, characteristic of CBr stimulation, was observed. Increasing ISP from 50 to 100 mm Hg to selectively increase CBr activity also failed to affect airway patency, but did induce a fall in MABP. Returning ISP from 100 to 50 mm Hg increased R_L transiently. These findings suggested that CBr have no tonic influences on the lower airways. This conclusion was supported by another series of experiments, in which AII was used to examine the effects of increasing systemic blood pressure on airway tone. Although AII produced significant bronchoconstriction and rapid, shallow breathing, these effects were not related to its pressor actions, and were not altered by baroreceptor denervation. Similarly, pressor agents such as phenylephrine and nordefrin had little effect on airway patency. Furthermore, the effects of NA on airway patency were not related to its pressor actions.

The lack of a CBr influence on airway tone, does not preclude stimulation of these receptors from altering airway patency under certain circumstances. Baroreceptor stimulation reduces MABP in this species by a withdrawal of sympathetic tone (H.S. Sun, personal communication). It was postulated that the withdrawal of sympathetic tone may explain the bronchoconstrictor responses to CSN stimulation and NSAID and TZ. In normal guinea pig airways, bronchodilator sympathetic tone is absent; thus, CBr stimulation should not influence airway tone in these animals. Some animals may present with sympathetic bronchodilator tone and CBr stimulation in this subset could induce bronchoconstriction. This possibility was examined by comparing the effects of propranolol in normal and ovalbumin-sensitized guinea pigs. In untreated normal animals, propranolol, had no effect on bronchomotor tone. By contrast, in ovalbumin-sensitized animals, propranolol induced bronchoconstriction. It is significant that the bronchoconstrictor responses to propranolol and that induced by NSAID and TZ (41) were reflected in increases in R_T which were of similar magnitude. The effects of CBr stimulation on airway patency should be explored in ovalbumin-sensitized guinea pigs to demonstrate that CBr-induced withdrawal of sympathetic tone can induce bronchoconstriction.

Retrograde injection of NaCN into the sinus to stimulate CCr did produce bronchoconstriction. The significance of this increase is questionable in light of its small size. It was suggested that increases in R_L produced when ISP was returned from 100 to 50 mm Hg was due to a transient increase in CCr activity. Although CCr activity is reported to transiently change with ISP in other species (46), it has not been established in the guinea pig. The experiment to demonstrate this has not been performed and may be impossible, in light of the inability to record from single receptor units.

It should be noted that NaCN stimulates other chemoreceptor centers. The ventilatory responses to NaCN injected retrogradely into the sinus were attributed to a reflex effect of local stimulation of CCr because of their time course, but the response was not fully characterized. However, NaCN injected systemically and intraaortically has no effect on either R_T and E_T (98). This further suggests that the responses to NaCN injected retrogradely in to the sinuses are due to a local effect; however, the effects of CSN section, decerebration, and ganglionic blockade on NaCN-induced increases in $\dot{V}e$ and R_L should be studied to demonstrate that the effects of NaCN were due to local stimulation of the carotid chemoreflex.

The final study reported in this thesis was performed to determine if stimulation of α-adrenoceptors could induce an increase in R_L. To examine this hypothesis, the effects on airway patency of NA and two α-adrenoceptor agonists were examined in normal and ovalbumin-sensitized guinea pigs. Sympathetic stimulation induced bronchodilation and modest bronchoconstrictor responses in normal animals, but only the latter was present in the ovalbumin-sensitized guinea pigs. It is unclear whether the bronchoconstrictor response is due to an increased sensitivity of the muscle to adrenoceptor stimulation or to the hyperresponsive state present in ovalbumin-sensitized animals. The relatively modest bronchoconstrictor response to NA in both normal and ovalbumin-sensitized guinea pigs suggests that α-adrenoceptors do not play a significant role in maintaining airway patency. Moreover, neither yohimbine or prazosin either alone or in combination had any effect on airway patency, indicating that α-adrenoceptors have no tonic influence on the airways in normal guinea pigs.

There are a number of questions as to how sympathetic system acts to affect airway patency. It is currently accepted that, in guinea pigs, the airways are not innervated by noradrenergic afferents and that β- and α-adrenoceptors in the lower airways respond to circulating catecholamines (277). Recent ultrastructural studies have established the presence of an adrenergic innervation to bronchial smooth muscle in humans (69, 193) and dogs (E.E. Daniel, personal communication) and suggest a similar innervation may exist in guinea-pig airways. There is some evidence in favor of such innervation. Electrical stimulation of sympathetic outflow to the airways induces bronchodilation in guinea pigs (8). In keeping with current beliefs, the bronchodilation was ascribed to an overflow of NA from well-innervated vasculature (8). Clearly, the presence of sympathetic innervation to bronchial smooth muscle could also explain the phenomenon. However, the lack of a response to propranolol, prazosin, and yohimbine suggests that the sympathetic system may have little functional control over guinea-pig airways.

In conclusion, CBr exert no tonic influences on the airways in guinea pigs. Chemoreceptor stimulation induces a slight increase in bronchomotor tone and appears to have little role in the regulation of the airways. Noradrenaline is both a bronchodilator and a bronchoconstrictor in guinea-pig airways. The latter effects of NA are minor in healthy and sensitized animals, and its significance is questionable.

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